University of Alberta

Reclamation of Diesel Fuel Contaminated Sites in Northern Alberta

by

Nancy Ann McIntyre

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the

requirements for the degree of Master of Science

in

Land Reclamation and Remediation

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Abstract

This study evaluated the relative effectiveness of natural attenuation and in-situ biostimulation of soil contaminated with weathered diesel fuel at two remote locations (Pony Creek and Birch Mountain) in the boreal forest of northeastern Alberta. Treatments included control unfertilized, control fertilized (N-P-K), contaminated unfertilized and contaminated fertilized (N-P-K) from 1998 to 2000. At Pony Creek, hydrocarbon levels decreased 4% with natural attenation and significantly (46%) with biostimulation. Hydrocarbon levels did not decrease at Birch Mountain during 1998 to 2000, possibly due to low soil moisture, but decreased 30% in 2001 with biostimulation. Fertilizer application stimulated an increase in soil microorganism populations and vegetation canopy cover and altered taxa composition of both microorganisms and vegetation canopy. *Pasturella multocida*, an aerobic bacteria that has not been previously identified as a hydrocarbon degrader, was isolated from the contaminated soil. *Agrostis scabra* Willd. (tickle grass) increased significantly with fertilizer application in the contaminated treatments.

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A final footnote
For those who are gone
For Linda and for Joyce
This chapter has ended
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Dedication

For the lady I love; this one is for you!

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1. Reclamation Of Diesel Fuel Contaminated Sites In Northern Alberta: Introduction

1.1 Background

There are approximately 15,000 fuel storage tanks in Alberta, where this research was conducted (Petroleum Tank Management Association of Alberta 2000). The tanks may be utilized for dispensing gasoline or diesel fuel at service stations, on farms, for generators at remote locations or backup power to other locations. Approximately 35% of these tanks will have one or more spills within their operational life (Petroleum Tank Management Association of Alberta 2000). Spills can occur when fuel is delivered or dispensed, through mechanical failures, breaks in fuel lines or tank corrosion.

Selection of treatment options for reclamation of diesel fuel contaminated soils is affected by legislation. Reclamation criteria for hydrocarbon levels in soil and, in some cases, acceptable processes for meeting regulatory levels vary considerably with jurisdiction. In Alberta the standards were 1000 ppm total petroleum hydrocarbons (TPH) for residential, 2000 ppm for commercial and 5000 ppm for industrial uses (Alberta Environmental Protection 1994) when this research was initiated. After initiation of this research, new guidelines were issued (Alberta Environment 2001), based on the Canadian Council of Ministers for the Environment (CCME) guidelines for reclamation of petroleum contaminated soil. The regulatory limit for diesel fuel contamination is 800 ppm TPH for fine-grained topsoil in parkland under the revised Alberta guidelines.

When hydrocarbon contamination of soil occurred in the past, the response was either soil removal or "leaving it to nature". Current research is focused on engineered approaches, biostimulation and natural attenuation. Engineered approaches involve addition of air, water, nutrients, bacteria or surfactants into injection wells and removal of fuel and/or fuel vapor through recovery wells. Biostimulation includes addition of nutrients, bacteria and/or surfactants on the soil surface or incorporated into the soil with depth. Natural attenuation relies on the inherent capability of microorganisms and vegetation for self reclamation and restoration of an ecosystem after disruption by hydrocarbon contamination. Depending on local legislation, some or all of these, and

other processes not described, may be used to achieve reductions in soil hydrocarbon levels.

Biodegradation of hydrocarbons occurs in the environment at a relatively low rate (Atlas and Bartha 1998). The rate of biodegradation varies with the type and size of the hydrocarbon molecule. Diesel fuel includes linear alkanes, branched alkanes, cycloalkanes and aromatic rings (Atlas 1981). Straight alkanes are typically easier to degrade than branched alkanes and aromatic compounds. As hydrocarbons degrade, remaining compounds are more recalcitrant to biodegradation processes.

1.2 Diesel Fuel Contamination and Biodegradation

Extensive literature exists on the impact of hydrocarbons and biodegradation of hydrocarbon contaminated soils; some literature also exists on diesel fuel contaminated soils and biostimulation. Detailed literature reviews of the impact of hydrocarbons and/or fertilizer on soil microorganisms and vegetation are provided in Chapters 2 and 3, respectively. A detailed literature review of biostimulation of hydrocarbon contaminated soils is provided in Chapter 2.

Fuel spills can affect soil quality as a growth medium for plants and microorganisms or affect water quality for drinking or aquatic life. Diesel fuel in soil is toxic to some microorganisms and indirectly affects others through reduction of aeration and altering the carbon to inorganic nutrient balance in the soil (Chapter 2). Overall, soil microorganism populations usually increase following a hydrocarbon spill; species dominance may be altered to favor those capable of hydrocarbon degradation. Vegetation can be damaged by diesel fuel through direct contact toxicity or indirectly through oxygen deprivation of plant roots, reduced availability of nutrients or toxic metabolites produced during the biodegradation process (Chapter 3). Live vegetation cover usually decreases following a diesel fuel spill, however, some species and genotypes within species of plants are more tolerant of hydrocarbon contamination.

Nitrogen and phosphorus supplements are often utilized to re-establish the nutrient level balance, increase soil microorganism populations and stimulate biodegradation of the contaminant hydrocarbons (Chapter 2). Biodegradation of hydrocarbons can occur over

a wide range of temperatures (Atlas 1981); although microorganism activity level is lower at reduced temperatures (Alexander 1999), biodegradation has been observed at soil temperatures down to approximately 0 °C.

1.3 Contributions of this Research

Most biostimulation studies have been conducted in a controlled laboratory setting, often with a single microorganism or with controlled applications of fuel in a field setting. Engineered solutions are more common than low technology approaches. Similarly, vegetation research has focused on phytotolerance or phytoremediation potential of specific species (typically agronomic) in planted monocultures.

There have been few studies on the effects of hydrocarbons and/or nutrients on mixed populations of microorganisms in-situ. No studies were identified which evaluated natural revegetation on hydrocarbon contaminated sites in the presence or absence of supplemental nutrients. Research has not addressed the complex interactions of a mixed population of microorganisms, native vegetation, weathered diesel fuel in soil and the cool climate in a boreal forest ecosystem.

1.4 Research Objectives

The objective of this research was to compare the effectiveness of natural attentuation and biostimulation for reduction of weathered diesel fuel in the boreal forest. Specifically,

1. To determine whether surface application of granular nitrate and phosphate fertilizer would enhance the rate of biodegradation to a depth of 70 cm (Chapter 2)
2. To determine effects of diesel fuel contamination and fertilizer application on bacteria populations and diversity (Chapter 2)
3. To determine separate and combined effects of diesel fuel contamination and fertilizer application on plant canopy cover and species richness of boreal forest sites with natural revegetation (Chapter 3)

1.5 References

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2. Reclamation Of Diesel Fuel Contaminated Sites In Northern Alberta: Natural Attenuation Versus Biostimulation

2.1 Introduction

Approximately 35% of all fuel storage tanks will have one or more spills within their operational life (Petroleum Tank Management Association of Alberta 2000). Many of these tanks contain diesel fuel that can damage existing vegetation, fill soil pores and reduce the number and generic composition of microorganisms. Selection of treatment options for reclamation of diesel fuel contaminated soils is affected by legislation. The target criteria for this study was set at 1000 ppm total petroleum hydrocarbons (TPH) based on Alberta government guidelines (Alberta Environmental Protection 1994) at the time this research was initiated.

Many microorganisms and a few plant species are capable of utilizing hydrocarbons, either as energy sources or for biosynthetic purposes. However, biodegradation of hydrocarbons occurs in the environment at a relatively low rate (Atlas and Bartha 1998) varying with type and size of the hydrocarbon molecule. Normal alkanes are typically easier to degrade than branched alkanes (Geerdink et al. 1996, Nocentini et al. 2000). Polycyclic aromatic compounds are the most difficult to degrade (Huessemann and Moore 1993, Wang et al. 1990, Guerin 2000). As hydrocarbon components are utilized, the remaining components are more recalcitrant to biodegradation processes (Novak et al. 1995). Diesel fuel includes linear alkanes, branched alkanes, cycloalkanes and aromatic rings (Atlas 1981, Geerdink et al. 1996). Few studies focus on weathered hydrocarbons (Novak et al. 1995, McMillen et al. 1995, Drake et al. 1995, Hatzinger and Alexander 1995, Davis et al. 1995); most were conducted in the laboratory with soil spiked with hydrocarbons and aged for up to two years.

Soil microorganism populations typically increase following hydrocarbon contamination, particularly if amended by inorganic nutrients (Jones and Edington 1968, Jones et al. 1970, Gudin and Syratt 1975, Jensen 1975a, Jensen 1975b, Llanos and Kjoller 1976, Sextone and Atlas 1977, Atlas 1978, Sextone et al. 1978, Sparrow et al. 1978, Dibble and Bartha 1979, Pinholt et al. 1979, McGill et al. 1981). However, no significant change

in microorganism population was observed by Odu (1972). Several studies have shown that following a hydrocarbon spill microorganism species dominance may be altered, with those capable of hydrocarbon degradation favored (Perry and Scheld 1968, Odu 1978, Pinholt et al. 1979). Petroleum in soil is toxic to some microorganisms and indirectly affects others through reduction of aeration and altering the carbon to inorganic nutrient balance in the soil. The net effect is to shift the overall population and species diversity of microorganisms (Jobson et al. 1979, Reynolds et al. 1999).

With hydrocarbon spills on soil, influx of carbon can disrupt the soil nutrient balance. Nitrogen and phosphorus supplements are often used to reestablish this nutrient balance and stimulate biodegradation of the contaminant hydrocarbons by soil microorganisms because these nutrients are important for bacteria. Nitrogen is an essential element for cell protein synthesis (Harder et al. 1991, van Eyk 1997) and phosphorus is essential for nucleic acid synthesis (Harder et al. 1991, Tisdale et al. 1993, van Eyk 1997). The ideal soil C:N ratio for some microorganisms is 10:1 (Jobson et al. 1974).

The effects of nutrient addition on hydrocarbon degradation in soil have been studied for nearly 30 years (Jobson et al. 1974, Evans et al. 1991, Bell et al. 1995, Davis et al. 1995, Guerin 2000). The results are generally positive although some studies have not demonstrated a significant effect (Walworth and Reynolds 1995). Recommended levels of nitrogen and phosphorus in soil vary from C:N:P of 100:60:6 to 100:0.5:0.1 (Wang et al. 1990, Evans et al. 1991, Harder et al. 1991, Huessemann and Moore 1993, Faessler et al. 1994, Mills and Frankenburger 1994, Margesin and Schinner 1997, Kirchmann and Ewnetu 1998, Graham et al. 1999). In a laboratory study, Harder et al. (1991) determined that the rate of hydrocarbon biodegradation was higher with both nitrate and phosphate added than with no nutrient addition or with only nitrate or phosphate addition. Both nitrogen and phosphorus are required for biodegradation, an over or under supply of either nutrient can reduce remedial efficiency (Graham et al. 1999).

Aerobic bacteria degrade hydrocarbons at a higher rate than anaerobic bacteria. Aeration or addition of a solid chemical oxygen source is frequently conducted in laboratory and field studies to provide oxygen to facultative bacteria and improve degradation rates of hydrocarbons (Evans et al. 1991, Bell et al. 1995, Davis et al. 1995,

Guerin 2000). Since higher oxygen concentrations increase the rate of biodegradation of hydrocarbons (Bonin and Bertrand 2000), biodegradation rates should be higher at shallow depths where oxygen levels are higher. However, only three field studies were identified where hydrocarbon levels were recorded at different depths (Grundmann and Rehm 1991, Bell et al. 1995, Davis et al. 1995). In each of these studies, the soils were oxygenated.

Soil temperature affects microorganism activity level (Alexander 1999) with the rate of hydrocarbon biodegradation in soil decreasing at reduced temperatures (Walworth and Reynolds 1995). In a review article, Atlas (1981) indicated that although biodegradation of hydrocarbons can occur over a wide range of temperatures, various studies have demonstrated hydrocarbon degradation up to an order of magnitude faster at 20 to 30 °C than at 0 to 5 °C. Biodegradation rates, although slower at lower temperatures, have been observed at soil temperatures down to approximately 0 °C (Maliszewska-Kordybach 1992, Ong et al. 1994, Sayles et al. 1995, Simpkin et al. 1995).

Most biodegradation studies have been conducted in a controlled laboratory setting. Of the studies reviewed, approximately two-thirds were conducted in the laboratory, generally with fuel added to the soil (Wang et al. 1990, Grundmann and Rehm 1991, Mills and Frankenburger 1994, Walworth and Reynolds 1995, Margesin and Schinner 1997). Two of the field studies used controlled applications of hydrocarbons (Jobson et al. 1974, Cansfield and Racz 1978); the remainder used spills existing prior to the research (Evans et al. 1991, Bell et al. 1995, Davis et al. 1995, Sayles et al. 1995, Simpkin et al. 1995). All field studies on existing spills were provided oxygen supplements. Little research was found which investigated surface in situ treatment. Research conducted in the 1970s on in situ treatment of oil spiked soil in Alberta and the Northwest Territories (Jobson et al. 1972, Jobson et al. 1974, Westlake et al. 1978) demonstrated enhanced degradation of oil with surface application of a urea-phosphate (27-27-0) fertilizer blend. A more recent study by Margesin and Schinner (2001) demonstrated enhanced degradation of diesel fuel spiked soil in an alpine region with application of 15-15-15 N:P:K fertilizer.

Although research to date has been valuable for improving our understanding of the contribution of microorganisms to the biodegradation process and details of the

biochemical pathways, it generally does not address the complex interactions of a mixed population of microorganisms, weathered diesel fuel in soil and the cool climate in a boreal forest ecosystem. Nearly all research is limited to one specific compound, often one specific organism and generally under controlled conditions in a laboratory setting or a simulated scenario in a field setting. Some research focuses on proprietary techniques, proprietary nutrients or proprietary organisms. Engineered solutions are more common than low technology approaches. There have been few studies on the effects of hydrocarbons and/or nutrients on mixed populations of microorganisms in situ.

2.2 Research Objectives

The objective of this research was to compare the effectiveness of natural attenuation and biostimulation for reduction of weathered diesel fuel in the boreal forest. Specifically, the intent was to determine whether surface application of granular nitrate and phosphate fertilizer would enhance the rate of biodegradation to a depth of 70 cm. A second objective was to determine effects of diesel fuel contamination and fertilizer application on bacteria populations and diversity.

2.3 Materials and Methods

2.3.1 Site Descriptions

The research was conducted at two TELUS communication tower sites in northeastern Alberta, Pony Creek (SW 28-80-8-W4, 80 km southeast of Fort McMurray) and Birch Mountain (SW 24-100-12-W4, 100 km northwest of Fort McMurray). Both are remote locations, generally accessed by helicopter. No permanent residences are present on or near the sites, although an Alberta Forestry fire lookout tower is occupied during the summer months at Birch Mountain. Diesel fuel generators provide power; fuel is transported to the site via a winter ice road.

Pony Creek is relatively flat with a gradual northeast slope (5%). Birch Mountain is elevated and has steep slopes (17%) to the east and west. The surrounding areas are

boreal forest. Vegetation in the previously cleared tower areas was dominated by native grass, forb and shrub species. Mean annual temperature is approximately 0 °C at Pony Creek and -2 °C at Birch Mountain (Environment Canada 2001); May to August mean temperature is 12 °C (normal summer range is 3 to 21 °C at Pony Creek and 3 to 19 °C at Birch Mountain). Pony Creek total annual precipitation (snow and rain) is 460 mm; mean May to August rainfall is 300 mm. Birch Mountain total annual precipitation is approximately 380 mm; mean May to August rainfall is 290 mm.

In 1990, at Pony Creek, approximately 8,000 L of diesel fuel was spilled and contaminated the soil northward from the diesel generator building; the contaminated area was approximately 16 x 25 x 0.7 m depth. No remedial actions were undertaken at the time of the spill. In 1979, at Birch Mountain, approximately 25,000 L of diesel fuel spilled from the generator building and contaminated the soil down slope to the east and southeast of the generator building; the contaminated area was approximately 20 x 20 x 1 m depth. In an attempt to control the spill, trenches were excavated and absorbent mats were placed in the trenches. In 1989/1990, an additional 1,000 L of diesel fuel was spilled in the same area.

2.3.2 Experimental Design and Treatments

The research was based on a split plot design. There were four treatments: control unfertilized, control fertilized, contaminated unfertilized and contaminated fertilized. Fertilized treatments were located to avoid possible runoff onto unfertilized areas. At Pony Creek, each treatment had five 8 by 5 m (40 m^2) replicate plots (Figure A.1). At Birch Mountain, each treatment had five 3.5 by 3 m (10.5 m^2) replicate plots; three additional 1.3 by 2.2 m (2.9 m^2) (data not shown) contaminated unfertilized and fertilized plots were located on a steeper slope below the other fertilized treatments (Figure A.2).

In summer 1998, fertilizer was applied to each fertilized treatment at a rate to achieve 336 kg ha^{-1} equivalent of nitrogen. The fertilizer was a slow-release 20-3-4 (nitrogen-phosphorus-potassium) blend. This rate was chosen to stimulate microorganism populations without damaging vegetation. In fall 1999, 34-0-0 fertilizer was applied at a rate to achieve 672 kg ha^{-1} equivalent nitrogen. This higher rate was to compensate for nitrogen utilization by vegetation and assess whether vegetation would tolerate the

higher rate. In 2000 fertilizer was applied at a rate to achieve 672 kg ha^{-1} equivalent of nitrogen. The fertilizer was a 4:1 mixture of 34-0-0 and 11-51-0. At Pony Creek in the contaminated fertilized treatment, holes were augered to a depth of approximately 1 m on a 1 m grid; half the fertilizer was placed in the holes and half on the surface. In the contaminated unfertilized treatment, fertilizer was surface spread only. At Birch Mountain, both contaminated treatments were treated with holes and fertilizer.

2.3.3 Soil and Meteorological Sampling and Analyses

Site reconnaissance and investigative soil sampling were conducted in early summer 1998 to determine the three-dimensional extent of contamination and boundaries for treatments. Soil samples were taken in summer and fall 1998, spring and fall 1999, fall 2000 and fall 2001. Interim results were used to assess progress and determine fertilizer requirements (data not shown). Soil was hand augered near the center of each plot to a depth of 70 cm, at least 15 cm from previous sampling locations. Samples were collected at 10 cm depth intervals and packed into labeled 125 ml glass jars. The auger was scraped to remove soil between samples.

Enviro-Test Laboratories (ETL) in Edmonton conducted all soil moisture, total extractable hydrocarbon (TEH) and gas chromatograph (GC) analyses. Soil moisture was gravimetric; oven dry at 105 °C. TEH concentration was determined by dichloromethane (DCM) extraction as a summation of the hydrocarbon contamination from the C_{11} to C_{30} range against a calibrated diesel standard. Methods followed a modified Environmental Protection Agency (EPA) SW-846 Method 3450 or 3580/8000 (Enviro-Test Laboratories 2002) (Appendix B.1).

Soil particle size distribution (sand, silt, clay) and soil water holding capacity (0.33 bar, 15 bar) were determined from Fall 1998 samples at the University of Alberta (Carter 1993). Soil analyses were conducted on Fall 2000 samples for available nitrate, phosphate, potassium and sulfate, total carbon, hydrogen ions in solution (pH), electrical conductivity (EC), cation exchange capacity (CEC) and sodium adsorption ratio (SAR) based on CSSS 21.4, CSSS 19.4, modified Kelowna extract, APHA NO3, CSSS 8.4 and CSSS 9.2 methods, respectively (Enviro-Test Laboratories 2002) (Appendix B.2).

Meteorological data were obtained from Environment Canada (2001). Summer data for Pony Creek were averaged from Algar (1959 to 1990) and Conklin (1954 to 1990) stations (approximately 30 km east and 30 km south, respectively); annual data were from the Fort McMurray airport (1944 to 1990) (approximately 80 km northwest). Summer meteorological data for Birch Mountain (1960 to 1990) came directly from the site; annual data were from the Fort Chipewyan airport (1967 to 1990) (approximately 100 km north).

2.3.4 Microbiological Analyses

Populations of aerobic, denitrifying, sulfate-reducing and iron-reducing bacteria were determined for control and contaminated treatments at all depths for selected plots in fall 1999 and fall 2000 (Appendix B.3). Plate counts of aerobic bacteria and most probable number (MPN) assessment of dilution tubes for anaerobic bacteria were used (Carter 1993). Population values were converted to a dry weight basis by adjusting for percentage moisture of the soil samples in the plot at the specific depth. In fall 2000, relative species dominance and nutritional diversity of aerobic bacteria were assessed through plate counts and Oxi/Ferm or Enterotube test kits for depths to 40 cm in selected plots at Pony Creek (Finegold and Baron 1986).

Morphologically distinct colonies from the counting level dilution of aerobic plate count agar plates were described, numbered and isolated on individual agar plates. If there was uncertainty whether two organisms were identical, both were described and plated. Morphologically identical organisms were isolated once within the same treatment if also identified at a different depth but were numbered, isolated and plated separately if in different treatments. After incubation at room temperature for 14 days, individual cultures were compared to original cultures on mixed plates. Cultures that were not identical or did not successfully colonize were replated from original cultures. If isolated cultures produced morphologically distinct colonies, they were renumbered and isolated onto individual plates. Individual cultures were replated onto numbered and dated plates every three weeks to maintain young, viable cultures. The process of replating, comparison to previous and original cultures and separation of morphologically distinct cultures was repeated three times. All cultures were retained throughout this process.

All organisms were tested for gram, oxidase and nutritional reactions with Oxi/Ferm test tubes. Uncertain or negative oxidase reaction tests were retested; uncertain or obscured gram stains were repeated. All slides were described morphologically and retained. Dr. Al Jobson, an experienced microbiologist, validated interpretation of gram stain results. Oxidase negative, gram negative bacteria were also tested with Enterotube II compartmented tubes. Repeated Oxi/Ferm and Enterotube tests were conducted for any bacteria that had no reactions or displayed atypical test responses.

Distinct organisms were identified by positive responses to gram staining, oxidase, anaerobic glucose, arginine, lysine, lactose, nitrogen gas, sucrose, indole, xylose, aerobic glucose, maltose, mannitol, phenylalinine, urea and citrate reactions. A diversity index was calculated based on the number of positive responses to the above reactions. Genera and species, where possible, were identified according to Oxi/Ferm (Becton Dickinson Microbiology Systems 1993a) and Enterotube (Becton Dickinson Microbiology Systems 1993b) interpretation manuals.

2.3.5 Statistical Analyses

All bacteria and hydrocarbon data were recorded in an MS Access database by depth. Statistical analyses were conducted with SAS version 8.1. A mixed general linear model was used to calculate least squares means, standard errors and differences of least squares means for each analysis. Error terms were calculated to include interactions between predictor variables. Statistical procedures followed Steele and Torrie (1980).

2.4 Results

Each research site was designed with the same site design, treatments and sampling approach. However, unique site characteristics resulted in distinct outcomes from the treatment program. The results and discussion for each site are addressed separately.

2.4.1 Soils

Soils were loam over sandy clay at Pony Creek and sandy loam at Birch Mountain (Table 2.1). Mean soil moisture levels from 1998 to 2001 at Pony Creek and Birch Mountain were approximately 82% and 80% of field capacity, respectively (Table 2.2). Soil moisture levels were consistent by depth for depths below 10 cm; soil at the 0 to 10 cm depth had 2 to 3 % higher moisture. Soil moisture levels were variable; during 1998 no moisture was measured in some samples. There were no significant differences between treatments for soil texture or soil moisture. Soils were acidic with low EC and SAR (Table 2.3). EC was significantly higher in treatments with fertilizer application. Available nutrients (nitrate and phosphate) were low. Nitrate, potassium and sulfate were significantly higher in the control fertilized treatment than in the control unfertilized treatment. Available nitrate, phosphate, potassium and sulfate were somewhat to significantly higher in the contaminated fertilized treatment than in the contaminated unfertilized treatment.

2.4.2 Bacteria Populations

2.4.2.1 Effect of contamination

Unfertilized control and unfertilized contaminated treatments were compared to determine contamination effects on bacteria populations. Populations of aerobic, iron reducing and sulfate reducing bacteria were 7 to 18% lower with contamination at Pony Creek (Tables 2.4, 2.5, 2.6 and 2.7); the denitrifying bacteria population was 146% higher. Results were less consistent at Birch Mountain where all bacteria populations (aerobic, denitrifying, iron reducing, sulfate reducing) were 17% lower to 5% higher with contamination (Tables 2.4, 2.5, 2.6 and 2.7). The effect was not consistent for all depths.

2.4.2.2 Effect of fertilizer application

To determine effects of fertilizer application on bacteria populations, unfertilized control and fertilized control treatments were compared. Populations of all bacteria (aerobic,

denitrifying, iron reducing, sulfate reducing) were 2 to 282% higher with fertilizer application at Pony Creek (Tables 2.4, 2.5, 2.6 and 2.7). Differences were significant for denitrifying (282%) and sulfate reducing bacteria (18%). This effect was consistent at all depths for aerobic and denitrifying bacteria and at shallow depths for iron and sulfate reducing bacteria. At Birch Mountain, populations of aerobic, denitrifying and iron reducing bacteria were 1 to 43% higher with fertilizer application. The population of sulfate reducing bacteria was 1% lower (Tables 2.4, 2.5, 2.6 and 2.7). Again, none of these effects were consistent with depth.

Populations of aerobic, iron reducing and sulfate reducing bacteria were 2% lower to 14% higher (15% at Birch Mountain) and denitrifying bacteria were significantly higher (180%) in the contaminated fertilized treatment than the control unfertilized treatment at both sites (Tables 2.4, 2.5, 2.6 and 2.7). At Pony Creek, populations of aerobic (15%) and denitrifying bacteria (180%) were significantly higher and iron and sulfate reducing bacteria were 10 to 16% higher in the contaminated fertilized treatment than in the contaminated unfertilized treatment. At Birch Mountain, populations of aerobic, iron reducing and sulfate reducing bacteria were 7 to 18% higher in the contaminated fertilized treatment than in the contaminated unfertilized treatment; the population of denitrifying bacteria was significantly higher (166%).

2.4.2.3 Identification

Most bacteria were not clearly identifiable using the Oxi/Ferm or Enterobacter II test kits. In fact many bacteria displayed atypical test responses (e.g., blue instead of yellow reaction for mannitol with the Oxi/Ferm test kit) (Table C.1). Identified bacteria in the control unfertilized treatment included *Acinetobacter lwoffii* and *Pseudomonas* spp. Biochemical reactions of some organisms matched nearly all reactions of *Agrobacterium* spp. or *Pasturella* spp. Identified bacteria in the contaminated unfertilized treatment included *Alcaligenes* spp. and *Pasturella multocida*. Biochemical reactions of some organisms matched nearly all reactions of *Pseudomonas* spp. Identified bacteria in the control fertilized treatment included *Actinobacter lwoffii* and *Pasturella multocida*. Biochemical reactions of some organisms matched nearly all reactions of *Achromobacter* spp., *Agrobacterium* spp., *Flavobacterium* spp. *Pasturella* spp. or *Pseudomonas* spp. The only identified bacteria in the contaminated fertilized treatment

was *Flavobacterium* spp. Biochemical reactions of some organisms matched nearly all reactions of *Achromobacter* spp., *Alcaligenes* spp., *Pasturella* spp. or *Pseudomonas* spp.

2.4.2.4 Biochemical reactions

Although most bacteria were not identified by genus, distinct aerobic organisms were characterized by their biochemical reactions. The number of distinct organism types was highest in the control unfertilized treatment (21), particularly at shallow depths and lowest in contaminated unfertilized (11) and control fertilized treatments (14). However, the number of distinct organism types in the contaminated fertilized treatment (18) was similar to the control unfertilized treatment and higher than the contaminated unfertilized treatment. The diversity of biochemical reactions within bacterial isolates was also highest in the control unfertilized treatment (Table 2.8).

Specific organism biochemical reactions characterized each treatment. Most organisms in the control unfertilized treatment were gram, oxidase, urea and citrate positive (Table 2.8). Most organisms in the contaminated unfertilized treatment were gram negative and oxidase positive. More organisms had a positive nitrogen gas and a negative urea reaction. Most organisms in the control fertilized treatment were gram, oxidase, lactose and urea positive. Fewer organisms had arginine, lysine and aerobic glucose reactions and more had a positive lactose reaction. Most organisms in the contaminated fertilized treatment were gram negative and oxidase positive. Significantly fewer organisms had arginine, citrate and urea reactions.

2.4.3 Hydrocarbons

2.4.3.1 Natural attenuation

TEH levels were 8% lower overall in 2000 than 1998 and lower at most depths in the contaminated unfertilized treatment at Pony Creek (Table 2.9, Figure C.1). Hydrocarbon levels from 20 to 50 cm decreased 44%. Hydrocarbon levels in fall 2000 met regulatory criteria in 26% of unfertilized plot depths and decreased more than 1000 ppm in an

additional 23% of unfertilized plot depths. There were no changes to the GC profile between 1998 and 2000 (Figures 2.1, 2.2).

At Birch Mountain, there was no consistent evidence of TEH level decreases in the contaminated unfertilized treatment; overall levels of contamination increased (Table 2.9). TEH levels in fall 2000 met regulatory criteria in 2% of the unfertilized plot depths and decreased more than 1000 ppm in an additional 20% of the unfertilized plot depths. At depths below 40 cm, TEH levels increased in most of the plots. On warm and sunny days diesel fuel was observed seeping out of the soil from the steep slope in some of the plots.

2.4.3.2 Biostimulation

Total hydrocarbon (TEH) levels were significantly lower overall (46%) and lower at all depths between spring 1998 and fall 2000 (Table 2.9, Figure 2.2) in the contaminated fertilized treatment at Pony Creek. The GC profiles from 2000 and 2001 demonstrate a loss of C_{10} to C_{15} hydrocarbons and a slightly steeper napthenic envelope (Figure 2.2). Hydrocarbon levels decreased 60% at depths from 20 to 70 cm. Hydrocarbon levels in fall 2000 met regulatory criteria in 26% of fertilized plot depths and decreased more than 1000 ppm in an additional 54% of fertilized plot depths. Between 2000 and 2001 in the fertilized only treatment, hydrocarbon levels decreased 32% (Table 2.10). This was reflected in a loss of normal alkanes and a steeper napthenic envelope on the GC profile (Figure 2.1). Between 2000 and 2001, in the fertilized with holes treatment, hydrocarbon levels decreased 29%. The napthenic envelope on the GC profile became steeper (Figure 2.2) between 2000 and 2001.

At Birch Mountain, there was some evidence of reduction of hydrocarbon levels between the years 1998 and 2000 in some of the fertilized plots from surface to 40 cm depths in plots in the contaminated fertilized treatment closest to the source of contamination (Table 2.9). Hydrocarbon levels in fall 2000 met regulatory criteria in 4% of fertilized plot depths and decreased more than 1000 ppm in an additional 27% of fertilized plot depths. At depths below 40 cm, TEH levels increased in most plots. Between 2000 and 2001 in the fertilized with holes treatment, hydrocarbon levels decreased 30% (Table 2.10).

2.5 Discussion

2.5.1 Soils

Soils with high sand or clay have been generally rated as poor candidates for reclamation in the boreal forest (Macyk et al. 1987). High percent sand was identified as a limiting factor for biodegradation success at low temperatures in Arctic soils (Mohn and Stewart 2000). Conversely, humic acid and clay increased the rate of biodegradation of phenanthrene (Ortega-Calvo and Saiz-Jimenez 2000). Therefore soil texture at Pony Creek is likely better for reclamation than that at Birch Mountain and may have impacted the rate of biodegradation with either natural attenuation or biostimulation.

Soil moisture is frequently identified as a limiting factor for reclamation processes (Dibble and Bartha 1979; Tiwari et al. 1987, Eaton and Patriquin 1989, Mahli et al. 1990, Van Gestel et al. 1993, Alexander 1999). Dibble and Bartha (1979) determined the optimal moisture level for biodegradation of oily sludges was 30 to 90% of soil water holding capacity. Tiwari et al. (1987) and Mahli et al. (1990) determined the optimal moisture level for denitrification was equal to or greater than field capacity. Moisture levels at both sites were suboptimal for reclamation. Undetectable moisture levels in some samples at Birch Mountain in 1998 would compromise bacteria survivability.

Soil pH, EC and SAR of the study sites are considered suitable for reclamation in the boreal forest. Alexander (1999) noted that the rate of biodegradation tends to be faster at moderate pH levels. Dibble and Bartha (1979) determined that optimal pH was 7.5 to 7.8, a higher level than at either research site. Nutrient levels, particularly nitrate and phosphate, are important limiting factors for biodegradation; recommended levels of C:N:P are 100:60:6 to 100:0.5:0.1 (Wang et al. 1990, Evans et al. 1991, Harder et al. 1991, Huessemann and Moore 1993, Faessler et al. 1994, Mills and Frankenburger 1994, Margesin and Schinner 1997, Kirchmann and Ewnetu 1998, Graham et al. 1999). Soil nutrient levels increased with fertilizer application at both research sites; however, available nitrate and phosphate remained low at Pony Creek and very low at Birch Mountain.

2.5.2 Bacteria Populations

2.5.2.1 Effect of contamination

Soil bacteria populations typically increase following hydrocarbon contamination (Jones and Edington 1968, Jones et al. 1970, Gudin and Syratt 1975, Jensen 1975a, b, Llanos and Kjoller 1976, Sextone and Atlas 1977, Atlas 1978, Sextone et al. 1978, Sparrow et al. 1978, Dibble and Bartha 1979, Pinholt et al. 1979, McGill et al. 1981). Results of this study are not fully consistent with most studies. The bacteria population (aerobic, iron reducing and sulfate reducing) in the contaminated unfertilized treatment was lower than the control unfertilized treatment at both research sites; however, the population of denitrifying bacteria was higher at both sites. Perhaps this effect is more evident immediately following the initial contamination than ten to fifteen years after the spill events, as in this study.

2.5.2.2 Effect of fertilizer application

Soil bacteria populations typically increase following nitrate and phosphate fertilizer application (Jobson et al. 1974, Evans et al. 1991, Bell et al. 1995, Davis et al. 1995, Guerin 2000). Results of this study support previous research. At both research sites, fertilized treatments (both control and contaminated) had higher total bacteria populations than unfertilized treatments. Inconsistent effects by depth and for denitrifying bacteria at Birch Mountain were likely as a result of limited stimulation by low nutrient levels that were not significantly higher following fertilizer application. Most nutrients likely leached through sandy soil, similar to effects observed by Mohn and Stewart (2000). Variable moisture levels likely also impacted denitrifying bacteria populations (Tiwari et al. 1987, Mahli et al. 1990).

2.5.2.3 Bacteria diversity

Research has shown soil microorganisms capable of hydrocarbon degradation will be favored following a hydrocarbon spill (Perry and Scheld 1968, Odu 1978, Pinholt et al. 1979). Probable genera of bacteria in contaminated soil at Pony Creek included *Achromobacter*, *Alcaligenes*, *Flavobacterium*, *Pasturella* or *Pseudomonas* spp. Of

these, *Achromobacter* (Cutright and Lee 1994), *Alcaligenes* (Guerin and Boyd 1995, Hill et al. 1996), *Flavobacterium* (Kampfer et al. 1993, Whiteley and Bailey 2000) and *Pseudomonas* (Whitman et al. 1998, McNally et al. 1999, Chayabutra and Ju 2000, Yuan et al. 2000) have been identified as hydrocarbon degraders. *Pasturella multocida* has not been indicated in any reviewed research as a potential hydrocarbon degrader.

Some researchers found species dominance of bacteria can be altered (Jobson et al. 1979, Xu and Johnson 1997, MacNaughton et al. 1999) by hydrocarbon contamination because petroleum is toxic to some organisms and alters the soil nutrient balance. This research supports changes to species dominance as a result of hydrocarbon contamination, probably in favor of hydrocarbon degraders. Tests to confirm whether the unidentified bacteria are hydrocarbon degraders were not conducted, however, some exhibit similar biochemical reactions to bacteria that are hydrocarbon degraders. Further, this research indicates that bacterial species dominance is altered by fertilizer application.

There has been limited research on the relationship between specific bacterial biochemical reactions and hydrocarbon degradation. Mannitol was identified by Piehler and Paerl (1996) as an inhibitor to diesel fuel degradation. Pichtel and Liskanen (2001) found a higher rate of diesel fuel degradation with N-P-K fertilizer than urea and Cervelli et al. (2000) found hydrocarbons inhibited urea hydrolysis in soil. Results of this study indicate that contamination and/or fertilizer application shift the species dominance of aerobic organisms from nutritional generalists to specialists. The limited percentage of organisms capable of utilizing urea in the contaminated treatments potentially explains less favorable results when urea fertilizer application has been utilized in other research studies to stimulate biodegradation.

2.5.3 Hydrocarbons

Successful natural attenuation of diesel fuel in soil is dependent on site characteristics (Tiwari et al. 1987, Mahli et al. 1990, Mohn and Stewart 2000). Results at Pony Creek demonstrate some reduction in hydrocarbon levels without addition of any nutrients to contaminated soil. However, given the initial level of contamination, an average loss of 4% a year would result in a very lengthy period of time for reclamation of this site. There

was no evidence of significant metabolization of normal alkanes or aromatic compounds.

Nutrient stimulation, often with some form of nitrogen or phosphorus, is frequently successful in stimulating biodegradation of hydrocarbon contaminated soil (Jobson et al. 1974, Evans et al. 1991, Bell et al. 1995, Davis et al. 1995, Guerin 2000). Few studies have demonstrated success in situ with diesel fuel in a cold climate without aeration (Margesin and Schinner 2001). Results at both research sites and unpublished results from this researcher at many similar sites during the same time period support the feasibility of surface application of fertilizer to increase the rate of biodegradation of weathered diesel fuel in a cold climate. Normal alkanes and light aromatic compounds are being metabolized. Initial limited success at Birch Mountain is likely due to several factors: sandier soil and limited vegetation cover allowing leaching of nutrients, low moisture levels, limited stimulation of bacteria populations and, most importantly, ongoing diesel fuel migration from the contaminated soil upslope of the treatment areas. Passive aeration and subsurface fertilizer application was not effective in increasing biodegradation rate relative to surface fertilizer application at Pony Creek.

2.6 Conclusions

The effectiveness of natural attenuation and biostimulation for reduction of weathered diesel fuel in a cold climate was strongly influenced by site conditions, specifically soil texture and soil moisture. At Pony Creek, natural attenuation demonstrated a steady low rate of biodegradation. Surface application of granular nitrate and phosphate fertilizer effectively increased the rate of biodegradation to a depth of 70 cm. At Birch Mountain, sandy soil and variable soil moisture between 1998 and 2000 may have limited early success of biodegradation.

More than 10 years after initial diesel fuel contamination, all bacteria populations except denitrifying bacteria at Pony Creek were lower than in the control. Fertilizer application stimulated an increase in bacteria populations, although this effect was inconsistent at Birch Mountain. Contamination and fertilizer application reduced bacteria population diversity. Specifically, fewer organisms had positive gram, arginine, citrate and urea reactions.

Pasturella multocida, an aerobic bacterium that has not been previously identified as a potential hydrocarbon degrader, was isolated from soil contaminated with diesel fuel; tests were not conducted to confirm biodegradation potential.

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Table 2.1. Soil texture at Pony Creek and Birch Mountain, 0 to 70 cm depth, September 1998.

Depth Interval (cm)	Sand (%)	Silt (%)	Clay (%)	Standard Error	Soil Texture
Pony Creek					
0 to 10	38	49	13	4	loam
10 to 20	38	48	14	4	loam
20 to 30	42	42	16	3	loam
30 to 40	54	27	18	3	sandy loam
40 to 50	57	20	22	1	sandy clay
50 to 60	59	19	22	1	sandy clay
60 to 70	55	23	21	3	sandy clay
Birch Mountain					
0 to 10	72	23	4	1	loamy sand
10 to 20	70	24	6	1	sandy loam
20 to 30	69	24	7	2	sandy loam
30 to 40	72	20	8	2	sandy loam
40 to 50	73	18	9	1	sandy loam
50 to 60	74	17	9	1	sandy loam
60 to 70	73	16	10	1	sandy loam

Values represent the least squares means of 2 replicates at 7 depths and the standard error.

Table 2.2. Gravimetric soil moisture (%) at Pony Creek and Birch Mountain, merged 0 to 70 cm depth, by sample time.

Sample Time	Pony Creek	Standard Error	Birch Mountain	Standard Error
July 1998[1]	14.9	0.3	10.5	1.3
September 1998[2]	11.6	0.8	7.3	1.3
May 1999[3]	14.6	0.3	11.8	1.0
September 1999[1]	13.2	0.4	12.0	0.3
August 2000[1]	15.1	0.3	14.1	0.8
September 2001[4]	14.7	0.4	11.2	1.6
Field Capacity (0.33 bar)[5]	19.2	0.6	14.5	0.4
Wilting Point (15 bar)[5]	6.3	0.2	4.1	0.2

[1]Values represent the least squares means of 4 treatments, 5 replicates at 7 depths and the standard error.
[2]Values represent the least squares means of 4 treatments, 2 replicates for control treatments, 5 replicates for contaminated treatments at 7 depths and the standard error.
[3]Values represent the least squares means of 4 treatments, 2 replicates for control treatments, 3 replicates for contaminated treatments at 7 depths and the standard error.
[4]Values represent the least squares means of 2 treatments, 2 replicates at 3 depths and the standard error.
[5] Values represent the least squares means of 4 treatments, 2 replicates at 7 depths and the standard error. September 1998.

Table 2.3. Soil chemical characterization at Pony Creek and Birch Mountain by treatment, merged 0 to 70 cm depth, August 2000.

Characteristic	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	Standard Error
Pony Creek					
Available Nitrate ($mg\ kg^{-1}$)	1.4 a	6.3 b	1.3 a	6.3 a	1.6
Available Phosphate ($mg\ kg^{-1}$)	7 a	7 a	8 a	7 a	1
Available Potassium ($mg\ kg^{-1}$)	56 a	67 a	54 a	67 a	8
Available Sulfate ($mg\ kg^{-1}$)	3 a	8 a	4 a	17 b	3
Total Carbon (%)	0.3 a	0.5 ab	0.5 ab	0.8 b	0.1
pH	4.8 a	4.8 a	4.8 a	4.9 a	0.2
Electrical Conductivity ($mS\ cm^{-1}$)	0.12 a	0.36 b	0.14 a	0.53 c	0.05
Cation Exchange Capacity ($meq\ 100\ g^{-1}$)	10.1 a	10.3 a	10.0 a	9.9 a	0.7
Sodium Adsorption Ratio	2.3 a	1.7 a	2.2 a	3.8 a	1.3
Birch Mountain					
Available Nitrate ($mg\ kg^{-1}$)	1.2 a	13.5 b	1.0 a	2.4 a	2.4
Available Phosphate ($mg\ kg^{-1}$)	2 a	2 a	3 a	2 a	1
Available Potassium ($mg\ kg^{-1}$)	18 a	28 b	26 b	33 b	2
Available Sulfate ($mg\ kg^{-1}$)	2 a	7 b	8 b	5 ab	2
Total Carbon (%)	0.7 a	0.9 a	1.4 a	1.4 a	0.4
pH	5.2 b	4.4 a	5.0 b	5.1 b	0.2
Electrical Conductivity ($mS\ cm^{-1}$)	0.18 a	0.60 b	0.17 a	0.26 a	0.09
Cation Exchange Capacity ($meq\ 100\ g^{-1}$)	5.5 a	6.3 a	4.9 a	5.4 a	0.8
Sodium Adsorption Ratio	2.6 b	1.4 a	2.5 a	1.4 a	0.3

Values represent the least squares means of 2 replicates at 7 depths and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) between treatments.

Table 2.4. Comparison of aerobic bacteria populations (log_{10} CFU g^{-1} dry soil) at Pony Creek and Birch Mountain, 0 to 70 cm depth, merged September 1999 and August 2000.

Depth Interval (cm)	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	Standard Error
Pony Creek					
0-10	6.30 ab	7.37 c	5.69 a	6.75 b	0.30
20-30	5.71 a	6.56 b	5.67 a	5.89 a	0.23
40-50	4.93 ab	5.23 b	4.42 a	5.53 b	0.26
60-70	5.02 a	4.95 a	4.68 a	5.45 a	0.50
Overall	5.49 ab	6.02 b	5.11 a	5.90 b	0.22
Birch Mountain					
0-10	6.47 a	6.73 a	6.38 a	6.45 a	0.30
20-30	6.20 a	6.04 a	5.46 a	5.96 a	0.27
40-50	4.47 a	5.29 ab	5.52 ab	5.92 b	0.43
60-70	5.09 ab	4.36 a	5.26 ab	5.95 b	0.45
Overall	5.56 a	5.60 a	5.65 a	6.07 a	0.23

Values represent the least squares means of 2 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD; $p \leq 0.05$) among treatments.

Table 2.5. Comparison of denitrifying bacteria populations (log_{10} MPN g^{-1} dry soil) at Pony Creek and Birch Mountain, 0 to 70 cm depth, merged September 1999 and August 2000.

Depth Interval (cm)	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	Standard Error
Pony Creek					
0-10	0.93 a	3.14 b	2.54 ab	1.36 ab	0.70
20-30	1.65 a	2.95 a	1.17 a	1.48 a	0.99
40-50	-0.16 a	2.35 b	1.02 ab	1.47 ab	0.61
60-70	0.00 a	0.88 a	1.27 ab	2.53 b	0.54
Overall	0.61 a	2.33 b	1.50 ab	1.71 b	0.42
Birch Mountain					
0-10	1.19 a	1.68 a	1.49 a	2.47 a	0.98
20-30	1.21 a	0.83 a	0.27 a	0.80 a	0.64
40-50	0.00 a	0.77 a	0.60 a	1.52 a	0.59
60-70	-0.16 a	-0.72 a	0.00 a	1.49 b	0.33
Overall	0.56 a	0.80 ab	0.59 a	1.57 b	0.41

Values represent the least squares means of 2 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD; $p \leq 0.05$) among treatments.

Table 2.6. Comparison of iron reducing bacteria populations (log_{10} MPN g^{-1} dry soil) at Pony Creek and Birch Mountain, 0 to 70 cm depth, merged September 1999 and August 2000.

Depth Interval (cm)	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	Standard Error
Pony Creek					
0-10	5.03 a	6.23 a	4.65 a	5.66 a	0.55
20-30	5.04 a	5.15 a	4.54 a	5.08 a	0.39
40-50	4.51 a	4.64 a	4.22 a	4.15 a	0.58
60-70	5.29 a	4.35 a	4.38 a	4.66 a	0.54
Overall	4.97 a	5.09 a	4.44 a	4.89 a	0.26
Birch Mountain					
0-10	4.78 a	6.55 b	4.94 ab	5.72 ab	0.56
20-30	5.23 a	4.80 a	4.52 a	5.44 a	0.71
40-50	4.54 a	4.70 a	5.05 a	5.06 a	0.71
60-70	4.95 a	4.10 a	4.56 a	6.13 a	0.71 0.51 0.51 0.51
Overall	4.87 ab	5.04 a	4.77 ab	5.59 b	0.34 0.31 0.31 0.31

Values represent the least squares means of 2 replicates at 4 depths and the standard error.
Different letters in a row indicate statistically significant differences (LSD; $p \leq 0.05$) among treatments.

Table 2.7. Comparison of sulfate reducing bacteria populations (log_{10} MPN g^{-1} dry soil) at Pony Creek, 0 to 70 cm depth, merged September 1999 and August 2000.

Depth Interval (cm)	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	Standard Error
Pony Creek					
0-10	5.15 ab	6.38 b	3.94 a	5.16 ab	0.72
20-30	4.46 ab	6.01 b	3.84 a	4.33 ab	0.58
40-50	4.12 a	5.14 a	3.73 a	4.55 a	0.41
60-70	4.74 a	4.27 a	3.61 a	3.44 a	0.46
Overall	4.61 b	5.45 c	3.78 a	4.37 ab	0.30
Birch Mountain					
0-10	5.03 a	5.24 a	4.73 a	5.32 a	0.70
20-30	5.15 a	5.35 a	4.30 a	4.72 a	0.68
40-50	4.60 a	4.49 a	4.10 a	4.90 a	0.40
60-70	4.40 b	4.02 ab	2.94 a	4.02 b	0.36
Overall	4.85 b	4.78 ab	4.02 a	4.74 ab	0.30 0.28 0.28 0.28

Values represent the least squares means of 2 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD; $p \leq 0.05$) among treatments.

Table 2.8. Comparison of biochemical reactions of aerobic bacteria at Pony Creek, 0 to 40 cm depth, August 2000, % of population.

Depth Interval (cm)	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	Standard Error
Oxidase	71 a	62 a	76 a	83 a	15
Citrate	69 b	27 a	43 ab	34 a	11
Urea	62 a	71 a	63 a	28 a	15
Gram	54 ab	58 b	32 ab	21 a	11
Lysine	39 b	4 a	37 b	14 ab	10
Aerobic Glucose	26 b	4 a	10 ab	15 ab	7
Arginine	24 b	0 a	20 ab	4 ab	7
Sucrose	24 a	38 a	26 a	26 a	9
Lactose	20 a	46 a	46 a	32 a	16
Xylose	17 a	12 a	10 a	0 a	8
Mannitol	16 a	4 a	10 a	8 a	7
Phenylalinine	13 b	0 a	0 a	0 a	4
Anaerobic Glucose	12 a	4 a	10 a	6 a	7
Nitrogen Gas	8 ab	0 a	22 b	10 ab	6
Indole	3 a	4 a	0 a	0 a	2
Maltose	0 a	0 a	10 a	15 a	6
Diversity Index	4 b	3 ab	2 a	3 ab	1

Values represent the least squares means of 4 depths and the standard error.
Different letters in a row indicate statistically significant differences (LSD; $p \leq 0.05$) among treatments.

Table 2.9. Comparison of hydrocarbon levels (ppm) by treatment by depth at Pony Creek and Birch Mountain, 0 to 70 cm depth, July 1998 and August 2000.

	1998		2000		
Depth Interval (cm)	Contaminated Unfertilized	Contaminated Fertilized	Contaminated Unfertilized	Contaminated Fertilized	Standard Error
Pony Creek					
0-10	5,684 a	3,712 a	7,570 a	7,602 a	2,998
10-20	11,302 a	15,190 a	11,749 a	11,266 a	5,349
20-30	5,658 a	13,620 b	3,260 a	3,483 a	2,139
30-40	3,020 a	9,340 b	1,620 a	3,042 a	957
40-50	2,760 a	10,240 b	1,540 a	3,940 a	1,336
50-60	1,800 a	11,540 b	1,680 a	4,900 a	1,679
60-70	1,680 a	10,900 b	1,500 a	5,371 a	1,848 1,848 1,848 1,962
Overall	4,558 a	10,649 b	4,131 a	5,858 a	1,851 1,851 1,851 1,868
Birch Mountain					
0-10	3,416 a	7,340 a	7,166 a	3,539 a	2,668
10-20	15,811 a	18,500 a	15,640 a	19,140 a	5,431
20-30	12,100 a	15,560 ab	18,040 a	20,200 b	2,982
30-40	16,800 a	16,200 a	17,000 ab	17,800 a	2,063
40-50	14,900 a	13,000 a	19,400 a	20,200 a	2,943
50-60	11,300 a	11,980 a	20,400 a	18,800 b	1,732
60-70	13,980 ab	10,420 a	19,200 a	25,000 c	2,893
Overall	12,615 a	13,286 a	16,692 bc	17,811 c	1,787

Values represent the least squares means of 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD; $p \leq 0.05$) among treatment-years.

Table 2.10. Comparison of hydrocarbon levels (ppm) by treatment by depth at Pony Creek and Birch Mountain, 0 to 70 cm depth, August 2000 and September 2001.

	2000		2001		
Depth Interval (cm)	Contaminated Fertilized	Contaminated Fertilized with Holes	Contaminated Fertilized	Contaminated Fertilized with Holes	Standard Error
Pony Creek[1]					
0-10	7,167 a	5,667 a	4,937 a	3,543 a	3,543
30-40	1,133 a	2,836 a	1,035 a	2,300 a	824
60-70	1,133 a	7,402 a	467 a	4,080 a	2,410
Overall	3,144 a	5,302 a	2,147 a	3,756 a	2,004
Birch Mountain[2]					
0-10		8,100 a		1,942 a	3,587
30-40		20,250 a		17,000 a	1,175
60-70		17,000 a		14,250 a	3,504
Overall		15,117 a		11,064 a	2,405

[1]Values represent the least squares means of 3 replicates and the standard error.
[2]Values represent the least squares means of 4 replicates and the standard error.
[2]At Birch Mountain, all replicates were fertilized with holes after 2000 sampling, 2000 data were merged.
Different letters in a row indicate statistically significant differences (LSD; $p \leq 0.05$) among treatment-years.

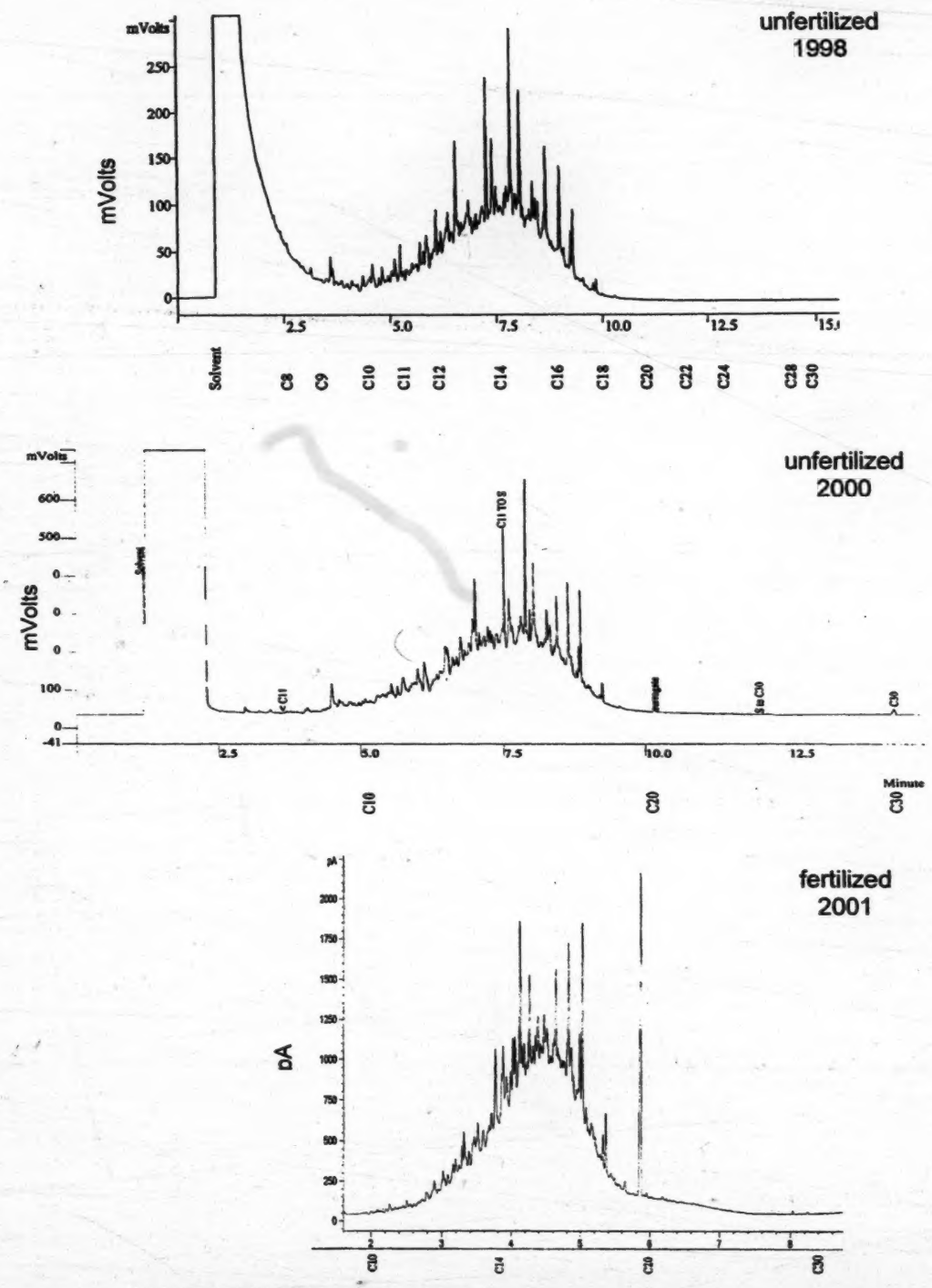

Figure 2.1. Pony Creek gas chromatograph profiles, unfertilized 1998, unfertilized 2000, fertilized 2001, aligned at C_{10} and C_{20} (Enviro-Test Laboratories).

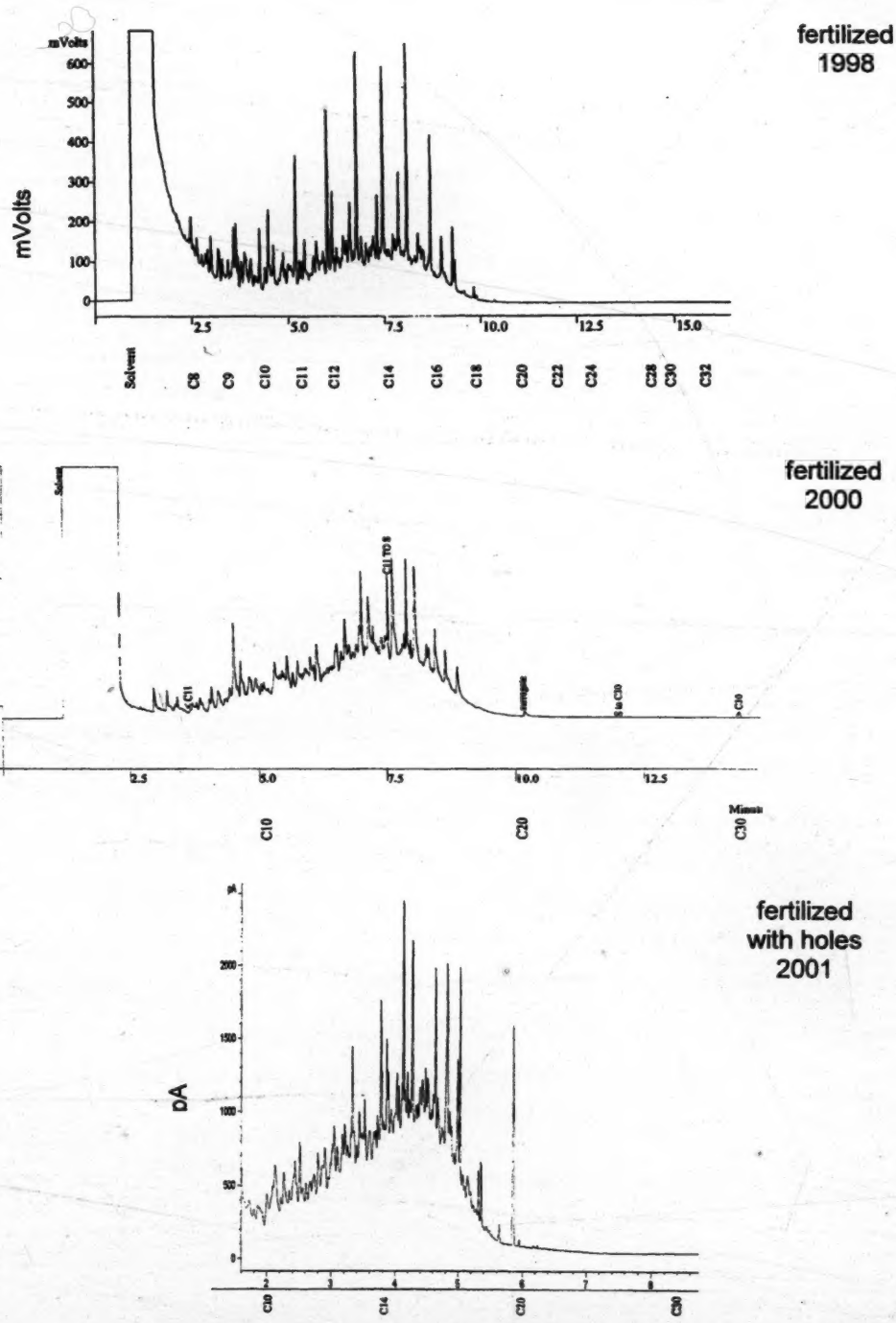

Figure 2.2. **Pony Creek gas chromatograph profiles, fertilized 1998, fertilized 2000, fertilized with holes 2001, aligned at C_{10} and C_{20} (Enviro-Test Laboratories).**

Chapter 3. Reclamation Of Diesel Fuel Contaminated Sites In Northern Alberta: Impact On Vegetation

3.1 Introduction

Approximately 35% of all fuel storage tanks will have one or more spills within their operational life (Petroleum Tank Management Association of Alberta 2000). Many of these tanks contain diesel fuel that can damage existing vegetation, fill soil pores and reduce the number and generic composition of microorganisms. Selection of treatment options for reclamation of diesel fuel contaminated soils is affected by legislation. The target criteria for this study was set at 1000 ppm total petroleum hydrocarbons (TPH) based on Alberta government guidelines (Alberta Environmental Protection 1994) at the time this research was initiated.

Hydrocarbons can be toxic on contact with plant roots and shoots (McGill et al. 1981). Monoaromatics are most toxic (Odu 1972), followed by olefins, naphthalenes and paraffins. Phytotoxicity is highest for low molecular weight and aromatic hydrocarbons (Chaineau et al. 1997). Nutrient depletion may affect plants if hydrocarbon degrading microorganisms consume reserves of nitrogen. Plants most affected by hydrocarbons include seedlings, annuals, mosses, lichens and plants with a large surface area or shallow root system (McGill and Nyborg 1975, McGill et al. 1981). Some species and genotypes within species of plants are more tolerant of hydrocarbon contamination (Rogers et al. 1996, Chaineau et al. 1997, Xu and Johnson 1997, Wiltse et al. 1998, Adam and Duncan 1999, Porta et al. 1999, Reynolds et al. 1999b). In one study (Rogers et al. 1996) root and shoot dry masses of *Poa alpina* L. (alpine blue grass) were higher in hydrocarbon contaminated soil than in uncontaminated soil.

One effect of carbon influx as a result of hydrocarbon spills on soil is disruption of the soil nutrient balance. Nitrogen and phosphorus supplements are often used to re-establish the balance of soil nutrients and stimulate biodegradation of the hydrocarbons.

Nutrients, particularly nitrogen and phosphorus, are frequently limiting factors for plant growth and development (Haag 1974, Shaver and Chapin 1980); potassium is less

frequently limiting (Shaver and Chapin 1995). Nitrogen and phosphorus cycling are slower at low temperatures that prevail in arctic ecosystems. Nitrogen and phosphorus are typically very low in forested or boreal (Vance and Entry 2000) and arctic ecosystems (Jonasson et al. 1996, Derry et al. 1999), with phosphorus a limiting nutrient in northern ecosystems (Chapin et al. 1978). Nitrogen, phosphorus and potassium (Chapin et al. 1975, Shaver and Chapin 1980, Shaver and Chapin 1995, Jonasson et al. 1996), nitrogen and phosphorus (Haag 1974), nitrogen and sulphur (Olson and Jacobsen 1999) or phosphorus fertilizer application alone (Chapin and Tryon 1982, Kielland and Chapin 1994) increases nutrient uptake and plant production. Chapin et al. (1975) determined high phosphorus fertilizer increased production more than high nitrogen fertilizer.

Specific plant species are more responsive to fertilizer application, including some grasses (Shaver and Chapin 1980, Berendse 1994, Derry et al. 1999, Olson and Jacobsen 1999) and mosses (Derry et al. 1999) and low nutrient adapted native species (Olson and Jacobsen 1999). Shaver and Chapin (1980) observed that *Calamagrostis canadensis* (Michx.) Beauv. (bluejoint) *Arctagrostis latifolia* (R. Br.) Griseb. (polar grass) and *Poa arctica* R. Br. (arctic bluegrass) are grasses typically found in high nutrient tundra sites.

Addition of fertilizer to soil can favour either vegetation or microorganisms. In studies by Zak et al. (1990) and Schimel et al. (1989) a greater percent of traced injections of nitrate and ammonium into the soil were incorporated into bacteria biomass than plant biomass in a Michigan hardwood forest and a California grassland. Jonasson et al. (1996) identified increased nitrogen and phosphorus in bacteria after fertilizer application and concluded nutrient immobilization could lead to competition with vegetation in arctic soils.

Addition of nutrients to hydrocarbon contaminated soil can result in competition between plants and bacteria. Xu and Johnson (1997) found lower plant nitrogen levels in hydrocarbon contaminated soils could be due to competition for nutrients with microorganisms. Reynolds et al. (1997) determined that plants without nutrient additions had reduced biodegradation rates in an arctic soil.

Some research has focused on phytoremediation of hydrocarbon contaminated soils. This is sometimes referred to as rhizosphere enhanced bioremediation (Reynolds et al. 1997). Aprill and Sims (1990) indicated three possible mechanisms for stimulation: improvement of soil physical and chemical properties, increased activity of soil microorganisms and increased contact between rhizosphere bacteria and contaminants. A fourth mechanism identified by Wild and Jones (1992) is direct uptake of contaminants.

Studies identified reduction of hydrocarbons on vegetated soils (Carman et al. 1998). Several (Aprill and Sims 1990, Reilley et al. 1996, Banks et al. 1997, Wiltse et al. 1998, Banks et al. 1999, Chaineau et al. 2000, Miya and Firestone 2000, Nedunuri et al. 2000, Hutchinson et al. 2001) demonstrated higher hydrocarbon degradation rates with vegetation (agronomics, grasses, legumes) than unvegetated soil in both laboratory and field. Vegetation and nutrient additions have demonstrated higher biodegradation rates than with nutrients alone (Reynolds et al. 1997, Reynolds et al. 1999a, 1999b).

Research to date has been valuable for improving understanding of the impact of fertilizer application on vegetation. However, research is limited on the response of native plant species, specifically nonwoody boreal forest species. Although there have been studies, primarily laboratory, on phytotolerance of specific species to hydrocarbons or phytoremediation potential of those species, research is often focused on agronomic species in monocultures. No studies evaluated natural revegetation on hydrocarbon contaminated sites in the presence or absence of supplemental nutrients.

3.2 Research Objectives

The objective of this research was to determine separate and combined effects of diesel fuel contamination and fertilizer application on canopy cover and taxa richness of naturally revegetated boreal forest sites.

3.3 Materials and Methods

3.3.1 Site Descriptions

The research was conducted at two TELUS communication tower sites in northeastern Alberta, Pony Creek (SW 28-80-8-W4, 80 km southeast of Fort McMurray) and Birch Mountain (SW 24-100-12-W4, 100 km northwest of Fort McMurray). Both are remote locations, generally accessed by helicopter. No permanent residences are present on or near the sites, although an Alberta Forestry fire lookout tower is occupied during the summer months at Birch Mountain. Diesel fuel generators provide power; fuel is transported to the site via a winter ice road.

Pony Creek is relatively flat with a gradual northeast slope (5%). Birch Mountain is elevated and has steep slopes (17%) to the east and west. The surrounding areas are boreal forest. Vegetation in the previously cleared tower areas was dominated by of native grass, forb and shrub species. Mean annual temperature is approximately 0 °C at Pony Creek and -2 °C at Birch Mountain (Environment Canada 2001); May to August mean temperature is 12 °C (normal summer range is 3 to 21 °C at Pony Creek and 3 to 19 °C at Birch Mountain). Pony Creek total annual precipitation (snow and rain) is 460 mm; mean May to August rainfall is 300 mm. Birch Mountain total annual precipitation is approximately 380 mm; mean May to August rainfall is 290 mm.

In 1990, at Pony Creek, approximately 8,000 L of diesel fuel was spilled and contaminated the soil northward from the diesel generator building; the contaminated area was approximately 16 x 25 x 0.7 m depth. No remedial actions were undertaken at the time of the spill. In 1979, at Birch Mountain, approximately 25,000 L of diesel fuel spilled from the generator building and contaminated the soil down slope to the east and southeast of the generator building; the contaminated area was approximately 20 x 20 x 1 m depth. In an attempt to control the spill, trenches were excavated and absorbent mats were placed in the trenches. In 1989/1990, an additional 1,000 L of diesel fuel was spilled in the same area.

3.3.2 Experimental Design and Treatments

The research was based on a split plot design. There were four treatments: control unfertilized, control fertilized, contaminated unfertilized and contaminated fertilized. Fertilized treatments were located to avoid possible runoff onto unfertilized areas. At Pony Creek, each treatment had five 8 by 5 m (40 m^2) replicate plots (Figure A.1). At Birch Mountain, each treatment had five 3.5 by 3 m (10.5 m^2) replicate plots; three additional 1.3 by 2.2 m (2.9 m^2) (data not shown) contaminated unfertilized and fertilized plots are located on a steeper slope below the other fertilized treatments (Figure A.2).

In summer 1998, fertilizer was applied to each fertilized treatment at a rate to achieve 336 kg ha^{-1} equivalent of nitrogen. The fertilizer was a slow-release 20-3-4 (nitrogen-phosphorus-potassium) blend. This rate was chosen to stimulate microorganism populations without damaging vegetation. In fall 1999, 34-0-0 fertilizer was applied at a rate to achieve 672 kg ha^{-1} equivalent of nitrogen. This higher rate was to compensate for nitrogen utilization by vegetation and assess whether vegetation would tolerate the higher rate. In 2000 fertilizer was applied at a rate to achieve 672 kg ha^{-1} equivalent of nitrogen. The fertilizer was a 4:1 mixture of 34-0-0 and 11-51-0. At Pony Creek in the contaminated fertilized treatment, holes were augered to a depth of approximately 1 m on a 1 m grid; half the fertilizer was placed in the holes and half on the surface. In the contaminated unfertilized treatment, fertilizer was surface spread only. At Birch Mountain, both contaminated treatments were treated with holes and fertilizer.

3.3.3 Soil and Meteorological Sampling and Analyses

Site reconnaissance and investigative soil sampling were conducted in early summer 1998 to determine the three-dimensional extent of contamination and boundaries for treatments. Soil samples were taken in summer and fall 1998, spring and fall 1999, fall 2000 and fall 2001. Interim results were used to assess progress and determine fertilizer requirements (data not shown). Soil was hand augered in approximately the center of each plot to a depth of 70 cm. Samples were collected at 10 cm depth intervals and packed into separately labeled 125 ml glass jars. The auger was scraped to remove soil between samples.

Enviro-Test Laboratories (ETL) in Edmonton conducted all soil moisture, total extractable hydrocarbon (TEH) and gas chromatograph (GC) analyses. Soil moisture was gravimetric, oven dry at 105 °C. TEH concentration was determined by dichloromethane (DCM) extraction as a summation of the hydrocarbon contamination from the C_{11} to C_{30} range against a calibrated diesel standard. Methods followed a modified Environmental Protection Agency (EPA) SW-846 Method 3450 or 3580/8000 (Enviro-Test Laboratories 2002) (Appendix B.1).

Soil particle size distribution (sand, silt, clay) and soil water holding capacity (0.33 bar, 15 bar) were determined from Fall 1998 samples at the University of Alberta (Carter 1993). Soil analyses were conducted on Fall 2000 samples for available nitrate, phosphate, potassium and sulfate, total carbon, hydrogen ions in solution (pH), electrical conductivity (EC), cation exchange capacity (CEC) and sodium adsorption ratio (SAR) based on CSSS 21.4, CSSS 19.4, modified Kelowna extract, APHA NO3, CSSS 8.4 and CSSS 9.2 methods, respectively (Enviro-Test Laboratories 2002) (Appendix B).

Meteorological data were obtained from Environment Canada (2001). Summer data for Pony Creek were averaged from Algar (1959 to 1990) and Conklin (1954 to 1990) stations (approximately 30 km east and 30 km south, respectively); annual data were from the Fort McMurray airport (1944 to 1990) (approximately 80 km northwest). Summer meteorological data for Birch Mountain (1960 to 1990) came directly from the site; annual data were from the Fort Chipewyan airport (1967 to 1990) (approximately 100 km north).

3.3.4 Vegetation Sampling

Vegetation was assessed in each treatment plot August 17 to 18,1998 and August 28 to 31, 2000 (Table C.2). Three 0.25 m^2 quadrats were sampled diagonally across each plot from southwest to northwest at Birch Mountain; five 0.25 m^2 quadrats were sampled at Pony Creek. At Pony Creek quadrats were located equidistant along the diagonal by sequentially stepping approximately 1 m from the corner of the plot or previous quadrat location. At Birch Mountain individual quadrats were located by sequentially stepping approximately 0.5 m from the corner of the plot or previous quadrat. Canopy cover (bare ground, live canopy cover, litter) and taxa composition by genera or species, were

visually assessed as a percentage of live canopy cover within each quadrat. Taxa composition was assessed for the upper canopy; taxa below the upper canopy were not recorded. In 2000, the maximum height of a plant within the quadrat was measured and overall plant vigour was visually assessed (data not shown).

Three transects on relatively undisturbed areas adjacent the research plots were surveyed in 1998 for plant taxa that could potentially naturally revegetate the research plots. At Pony Creek, transect 1 was located parallel to the unfertilized contaminated treatment 12 m south of the treatment area. Transect 2 was located 15 m south of transect 1 and transect 3 was located 9 m parallel to the unfertilized control treatment north of the treatment area. At Birch Mountain, transect 1 was located east of the research site, transects 2 and 3 west of the research site. Plant taxa in each transect were listed within a 0.25 m^2 quadrat at approximately 5 m intervals; additional taxa identified between quadrats were also listed. Taxa in the transition zone 2 to 3 m from the edge of the tall trees around the full perimeter of each site were also listed (data not shown).

3.3.5 Statistical Analyses

All vegetation data were recorded in an MS Access database at the quadrat level. Statistical analyses were conducted with SAS version 8.1. A mixed general linear model was utilized to calculate least squares means, standard error and differences of least squares means for each analysis. Error terms were calculated to include interactions between predictor variables. Statistical procedures followed Steele and Torrie (1980).

3.4 Results

Each research site was designed with the same treatments and sampling approach. However, unique site characteristics resulted in distinct outcomes from the treatment program; results and discussion for each site are addressed separately.

3.4.1 Soils

Soils were loam over sandy clay at Pony Creek and sandy loam at Birch Mountain (Table 3.1). Mean soil moisture levels from 1998 to 2001 at Pony Creek and Birch Mountain were approximately 82% and 80% of field capacity, respectively (Table 3.2). Soil moisture levels were consistent by depth for depths below 10 cm; soil at the 0 to 10 cm depth had 2 to 3 % higher moisture. Soil moisture levels were variable; during 1998 no moisture was measured in some samples. There were no significant differences between treatments for soil texture or soil moisture. Soils were acidic with low EC and SAR (Table 3.3, 3.4). EC was significantly higher in treatments with fertilizer application. Available nutrients (nitrate and phosphate) were low. Nitrate, potassium and sulfate were significantly higher in the control fertilized treatment than in the control unfertilized treatment. Available nitrate, phosphate, potassium and sulfate were somewhat to significantly higher in the contaminated fertilized treatment than in the contaminated unfertilized treatment.

3.4.2 Canopy Cover

3.4.2.1 Effect of contamination

Live canopy cover and litter cover were significantly lower (31 and 32%, respectively) and bare ground was significantly higher (275%) in the contaminated treatments than in the control treatments at Pony Creek in 1998 (Table 3.5a). In 2000, live canopy cover was 6% higher in the contaminated treatments and bare ground was not significantly different than in the control treatments. Grass and sedge cover, as a percentage of live canopy cover, were significantly higher in contaminated treatments than in control treatments in 1998 (48%) and 2000 (33%) and forb cover was significantly lower in 1998 (38%) and 2000 (43%) (Table 3.6a).

At Birch Mountain, live canopy cover did not differ in contaminated and control treatments in 1998; litter was significantly higher (121%) and bare ground was significantly lower (52%) in contaminated treatments than in control treatments (Table 3.5a). Shrub and tree cover, as a percentage of live canopy cover, was significantly

higher (105%) in contaminated treatments than in control treatments in 1998 but 48% lower in 2000 (Table 3.6a).

3.4.2.2 Effect of fertilizer application

There was no difference between live canopy cover in fertilized and control treatments at Pony Creek in 1998 (Table 3.5b). In 2000, live canopy coverwas significantly higher (23%) in fertilized than control treatments. Litter was significantly lower in fertilized than control treatments in 1998 (35%) and 2000 (88%). Bare ground was significantly higher in 1998 (145%) and 2000 (400%) in fertilized treatments than in control treatments. Shrub and tree cover, as a percentage of live canopy cover, was significantly lower in contaminated than control treatments in 1998 (83%) and 2000 (91%) (Table 3.6b). Grass cover was higher (8%) in contaminated than control treatments in 1998 and significantly higher (75%) in 2000. Forb cover was higher (24%) in contaminated than control treatments in 1998 and significantly lower (79%) in 2000.

At Birch Mountain, there were no significant differences in live canopy coveror litter cover between control and fertilized treatments in either 1998 or 2000 (Table 3.5b). Bare ground was 35% higher in fertilized than control treatments in 1998; in 2000 bare ground was significantly (50%) lower in fertilized treatments. There were no significant differences between control and contaminated treatments for any type of vegetation, as a percentage of live canopy cover, in 1998 (Table 3.6b). In 2000, shrub and tree cover (65%) and moss and lichen cover (62%) were significantly lower in contaminated than control treatments. Grass cover was significantly higher (97%) in contaminated than control treatments.

3.4.2.3 Effect of sample year

At Pony Creek in 1998, canopy cover in the control unfertilized treatment was split between live canopy coverand litter (Table 3.5c). Live canopy cover increased 23% between 1998 and 2000; litter decreased 12% and there was no bare ground in the control unfertilized treatment in 2000. Grasses and sedges, as a percentage of live canopy cover, decreased significantly (37%), forbs increased 14% and mosses and lichens increased significantly (300%) between 1998 and 2000 (Table 3.6c).

At Birch Mountain in 1998, canopy cover was 1/3 each of live vegetation, litter and bare ground in the control unfertilized treatment (Table 3.5c). Live canopy cover increased significantly (68%) between 1998 and 2000; litter decreased significantly (66%) and bare ground decreased 40%. Forbs, as a percentage of live canopy cover, decreased significantly (55%), mosses and lichens increased significantly (267%) and shrubs and trees increased 31% (Table 3.6c).

3.4.2.4 Interaction between contamination, fertilizer application and sample year

At Pony Creek in 1998, live canopy cover and litter cover in the contaminated fertilized treatment were significantly lower (17 to 37%) and bare ground significantly higher (175 to 780%) than in all other treatments (Table 3.7). Live canopy cover increased significantly (110 to 152%) between 1998 and 2000 in all treatments except the control unfertilized treatment. Live canopy cover in the control unfertilized treatment was significantly lower (34 to 53%) and litter significantly higher (68 to 95%) than all other treatments in 2000. There was no significant difference in bare ground between treatments in 2000. Grasses and sedges, as a percentage of live canopy cover, decreased significantly (37%) in the control unfertilized treatment, decreased 5% in the contaminated unfertilized treatment and increased significantly (46 to 108%) in both fertilized treatments between 1998 and 2000 (Table 3.8). Forbs decreased significantly (77 to 84%) in both fertilized treatments between 1998 and 2000. Mosses and lichens increased significantly (300%) in the control unfertilized treatment.

At Birch Mountain in 1998, bare ground was significantly higher (44 to 70%) in the control unfertilized treatment than in all other treatments (Table 3.7). Live canopy cover was lowest in the contaminated unfertilized treatment and significantly lower (8 to 23%) than all other treatments in 2000. Bare ground in the control fertilized treatment decreased significantly (80%) between 1998 and 2000. Grasses and sedges, as a percentage of live canopy cover, increased significantly (29 to 386%) and forbs decreased significantly (34 to 53%) in both fertilized treatments between 1998 and 2000 (Table 3.8). Mosses and lichens increased significantly (267%) between 1998 and 2000 in both control treatments.

3.4.3 Vegetation Taxa Richness and Major Taxa

3.4.3.1 Effect of contamination

At Pony Creek in 1998, prior to treatment, taxa richness was 20% higher in control than contaminated treatments (Table 3.9). Taxa richness decreased significantly (20%) in control treatments between 1998 and 2000. In 1998, live canopy cover of *Aster* L. (aster) (41%) and *Epilobium* L. (fireweed) (70%) were significantly higher in control treatments and *Poa* L. (bluegrass) cover was significantly (2600%) higher in contaminated than control treatments. In 2000, live canopy cover of *Achillea millefolium* L. (common yarrow) (100%), *Epilobium* spp. (88%), *Lycopodium* L. (club-moss) (100%) and *Vaccinium* L. (blueberry) (100%) was significantly higher in control than contaminated treatments and cover of *Agrostis scabra* Willd. (tickle grass) (833%) and *Poa* spp. (400%) was significantly higher in contaminated than control treatments.

At Birch Mountain taxa richness was significantly higher in 1998 (33%) and 2000 (17%) in control than in contaminated treatments (Table 3.10). In 1998, live canopy cover of *Calamagrostis* spp. (90%), *Plantago major* L. (common plantain) (500%) and *Taraxacum officinale* Weber (common dandelion) (400%) were significantly higher in control treatments than in contaminated treatments and the live canopy cover of *Arctostaphylos uva-ursi* (L.) Spreng. (common bearberry) (600%), *Cornus stolonifera* Michx (dogwood) and *Vaccinium* spp. were significantly higher in contaminated treatments than control treatments. In 2000, live canopy cover of *Alnus crispa* (Ait.) Pursh (green alder) (1700%) *Betula* L. (birch) (600%), *Poa* spp. (1900%) and *Taraxacum officinale* (600%) were significantly higher in control treatments than in contaminated treatments and *Carex* L. (sedge) (2800%) was significantly higher in contaminated treatments than in control treatments.

3.4.3.2 Effect of fertilizer application

Taxa richness at Pony Creek was significantly higher in control than fertilized treatments in 1998 (20%) and 2000 (67%) (Table 3.11). Taxa richness in fertilized treatments decreased significantly (67%) between 1998 and 2000. In 2000, after fertilizer application, live canopy cover of *Achillea millefolium* (300%), *Carex* spp. (91%),

Lycopodium spp. (300%) and *Vaccinium* spp. (300%) was significantly higher in control treatments and *Agrostis scabra* (317%), *Festuca rubra* L. (red fescue) (500%) and *Poa* spp. (250%) were significantly higher in fertilized than control treatments.

At Birch Mountain, taxa richness was significantly higher (33%) in control treatments than fertilized treatments in 2000 (Table 3.12). Taxa richness decreased 20% in fertilized treatments between 1998 and 2000. In 2000, after fertilizer application, live canopy cover of *Betula* spp. was significantly higher (500%) in the control treatments than in the fertilized treatments and *Agrostis scabra* (157%), *Festuca rubra* (500%) and *Poa* spp. (467%) cover was significantly higher in fertilized than control treatments.

3.4.3.3 Effect of sample year

Taxa richness at Pony Creek significantly decreased (20%) between 1998 and 2000 (Table 3.13). *Aster* spp. significantly decreased (62%) between 1998 and 2000, *Agrostis scabra* (79%) and *Carex* spp. (225%) significantly increased.

There was no significant change in taxa richness at Birch Mountain between 1998 and 2000 (Table 3.14). *Arctostaphylos uva-ursi* (400%), *Epilobium* spp. (65%), *Festuca rubra* (50%), *Rubus* L. (raspberry) (71%) and *Salix* L. (willow) (83%) decreased significantly between 1998 and 2000; *Agrostis scabra* (500%), *Carex* spp. (1400%) and *Poa* spp. (900%) increased significantly.

3.4.3.4 Interaction between contamination, fertilizer application and sample year

Taxa richness at Pony Creek in contaminated fertilized treatments was significantly lower (40 to 50%) in 1998 (Table 3.15, Figure C.3). Taxa richness in contaminated fertilized (33%) and control fertilized treatments (60%) decreased significantly between 1998 and 2000. In 1998, *Poa* spp. cover was significantly higher (1200 to 1300%) in contaminated than control treatments (Table 3.15, Figure C.4). *Achillea millefolium* (150%), *Carex* spp. (260%) and *Lycopodium* spp. (250%) cover increased significantly between 1998 and 2000 in the control unfertilized treatment; *Festuca rubra* decreased significantly (93%). In the contaminated unfertilized treatment, *Carex* spp. (367%) and *Trifolium hybridum* L. (alsike clover) (1100%) increased significantly; *Festuca rubra* (56%) and *Poa* spp. (65%) decreased significantly. *Achillea millefolium*, *Aster* spp. and

Trifolium hybridum were eliminated in fertilized treatments between 1998 and 2000. *Epilobium* spp. (300%) and *Festuca rubra* (106%) increased significantly (72% live canopy cover) between 1998 and 2000 in the control fertilized treatment; *Agrostis scabra* (422%) increased significantly (47% live canopy cover) in the contaminated fertilized treatment.

At Birch Mountain, taxa richness was significantly higher (29 to 43%) in the control unfertilized treatment (Table 3.16, Figure C.5) There were no consistent differences between treatments for any taxa in 1998. In 2000, in the contaminated unfertilized treatment *Cornus canadensis* L. (bunchberry) (67%) decreased significantly, *Festuca rubra* and *Salix* spp. were eliminated and *Carex* spp. increased (>100%). In the control fertilized treatment, *Calamagrostis* spp. (63%) and *Festuca rubra* (84%) decreased significantly; *Poa* spp. increased significantly (3300%) (Table 3.18, Figure C.6). In the contaminated fertilized treatment, *Arctostaphylos uva-ursi*, *Cornus canadensis*, *Cornus stolonifera* and *Salix* spp. were eliminated; *Agrostis scabra* (100%), *Carex* spp. (2000%) and *Festuca rubra* (186%) increased significantly between 1998 and 2000.

3.5 Discussion

3.5.1 Soils

Soils with high sand or clay have been generally rated as poor candidates for reclamation in the boreal forest (Macyk et al. 1987). Therefore soil texture at Pony Creek is likely better for revegetation than soil at Birch Mountain. Undetectable moisture levels in some samples at Birch Mountain in 1998 may have impacted vegetation.

Soil pH, EC and SAR of the study sites are considered suitable for reclamation in the boreal forest (Macyk et al. 1987). Nutrients, particularly nitrogen and phosphorus, are frequently limiting factors for plant growth and development (Haag 1974, Shaver and Chapin 1980). Nitrogen and phosphorus are typically very low in forested or boreal ecosystems (Vance and Entry 2000) with phosphorus a limiting nutrient in northern ecosystems (Chapin et al. 1978). Soil nutrient levels increased with fertilizer application at both research sites; however, available nitrate and phosphate remained low at Pony Creek and very low at Birch Mountain.

3.5.2 Canopy Cover

Results from this study, several years after the diesel fuel spill, are consistent with previous research in which most of the vegetation was impacted by hydrocarbon contamination (Odu 1972, Chaineau et al. 1997). Specific plant species are more responsive to fertilizer application, including some grasses (Shaver and Chapin 1980, Berendse 1994, Derry et al. 1999, Olson and Jacobsen 1999) and mosses (Derry et al. 1999) and low nutrient adapted native species (Olson and Jacobsen 1999). Phytotolerance of grasses and sedges at Pony Creek in contaminated treatments is consistent with previous research. Phytotolerance of shrubs and trees, particularly berry shrubs, to contamination at Birch Mountain is consistent with unreported observations by this researcher at similar sites.

Live canopy cover increased for fertilized treatments at both of the research sites consistent with previous studies in which nitrogen and phosphorus fertilizer application typically increased plant production (Haag 1974, Chapin et al. 1975, Shaver and Chapin 1980, Chapin and Tryon 1982, Kielland and Chapin 1994, Shaver and Chapin 1995, Jonasson et al. 1996). Increases in live canopy cover at both sites between 1998 and 2000 are likely a result of increased soil moisture, higher nutrients and decreases in hydrocarbon levels (Chapter 2). Overall, forbs (Pony Creek), mosses and lichens benefited from increased moisture levels.

3.5.3 Vegetation Taxa Richness and Major Taxa

Some species and genotypes within species of plants are more tolerant of hydrocarbon contamination (Rogers et al. 1996, Chaineau et al. 1997, Xu and Johnson 1997, Wiltse et al. 1998, Adam and Duncan 1999, Porta et al. 1999, Reynolds et al. 1999b). Vegetation most affected by hydrocarbons includes seedlings, annuals, mosses, lichens and plants with a large surface area or shallow root system (McGill and Nyborg 1975, McGill et al. 1981). Several years following the spills, perennial grasses, forbs and berry shrubs were present in the contaminated areas.

No research was identified which specifically evaluated taxa richness after hydrocarbon contamination. This research demonstrated higher taxa richness with lower initial levels of contamination and with decreasing levels of contamination.

Some vegetation is more responsive to fertilizer application, including some grasses (Shaver and Chapin 1980, Berendse 1994, Derry et al. 1999, Olson and Jacobsen 1999), mosses (Derry et al. 1999) and low-nutrient adapted native species (Olson and Jacobsen 1999). Shaver and Chapin (1980) observed that *Calamagrostis canadensis*, *Arctagrostis latifolia* and *Poa arctica* are grasses that typically are found in high nutrient sites in the tundra. *Arctagrostis latifolia* and *Poa hartzii* were among species with increased dominance after fertilizer application in a study by Derry et al. (1999). This research supports changes to live canopy cover to favor grasses as a result of fertilizer application.

No research was identified which specifically evaluated taxa richness after fertilizer application. Grasses and sedges were better adapted to higher levels of nutrients than forbs or berry shrubs; the resulting grass dominance as a percentage of live canopy cover in the canopy reduced overall canopy taxa richness.

3.6 Conclusions

More than ten years after the diesel fuel spills, live canopy cover was lower and bare ground was higher in the contaminated treatments. Fertilizer application stimulated an increase in live canopy cover, particularly the canopy cover of grasses and sedges as a percentage of live canopy cover. The percentage live canopy cover of *Agrostis scabra*, *Festuca rubra* and *Poa* spp. increased with fertilizer application. *Agrostis scabra* was favored by fertilizer application in the contaminated treatments.

3.7 References

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Table 3.1. Soil texture at Pony Creek and Birch Mountain, 0 to 70 cm depth, September 1998.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	SE	Soil Texture
Pony Creek					
0 to 10	38	49	13	4	loam
10 to 20	38	48	14	4	loam
20 to 30	42	42	16	3	loam
30 to 40	54	27	18	3	sandy loam
40 to 50	57	20	22	1	sandy clay
50 to 60	59	19	22	1	sandy clay
60 to 70	55	23	21	3	sandy clay
Birch Mountain					
0 to 10	72	23	4	1	loamy sand
10 to 20	70	24	6	1	sandy loam
20 to 30	69	24	7	2	sandy loam
30 to 40	72	20	8	2	sandy loam
40 to 50	73	18	9	1	sandy loam
50 to 60	74	17	9	1	sandy loam
60 to 70	73	16	10	1	sandy loam

Values represent the least squares means of 2 replicates at 7 depths and the standard error.

Table 3.2. Gravimetric soil moisture (%) at Pony Creek and Birch Mountain, merged 0 to 70 cm depth, by sample time.

Sample Time	Pony Creek	SE	Birch Mountain	SE
July 1998[1]	14.9	0.3	10.5	1.3
September 1998[2]	11.6	0.8	7.3	1.3
May 1999[3]	14.6	0.3	11.8	1.0
September 1999[1]	13.2	0.4	12.0	0.3
August 2000[1]	15.1	0.3	14.1	0.8
September 2001[4]	14.7	0.4	11.2	1.6
Field Capacity (0.33 bar)[5]	19.2	0.6	14.5	0.4
Wilting Point (15 bar)[5]	6.3	0.2	4.1	0.2

[1]Values represent the least squares means of 4 treatments, 5 replicates at 7 depths and the standard error.
[2]Values represent the least squares means of 4 treatments, 2 replicates for control treatments, 5 replicates for contaminated treatments at 7 depths and the standard error.
[3]Values represent the least squares means of 4 treatments, 2 replicates for control treatments, 3 replicates for contaminated treatments at 7 depths and the standard error.
[4]Values represent the least squares means of 2 treatments, 2 replicates at 3 depths and the standard error.
[5] Values represent the least squares means of 4 treatments, 2 replicates at 7 depths and the standard error. September 1998.

Table 3.3. Soil chemical characterization at Pony Creek by treatment, merged 0 to 70 cm depth, August 2000.

Characteristic	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	SE
Available Nitrate (mg kg^{-1})	1.4 a	6.3 b	1.3 a	6.3 a	1.6
Available Phosphate (mg kg^{-1})	7 a	7 a	8 a	7 a	1
Available Potassium (mg kg^{-1})	56 a	67 a	54 a	67 a	8
Available Sulfate (mg kg^{-1})	3 a	8 a	4 a	17 b	3
Total Carbon (%)	0.3 a	0.5 ab	0.5 ab	0.8 b	0.1
pH	4.8 a	4.8 a	4.8 a	4.9 a	0.2
Electrical Conductivity (mS cm^{-1})	0.12 a	0.36 b	0.14 a	0.53 c	0.05
Cation Exchange Capacity (meq 100 g^{-1})	10.1 a	10.3 a	10.0 a	9.9 a	0.7
Sodium Adsorption Ratio	2.3 a	1.7 a	2.2 a	3.8 a	1.3

Values represent the least squares means of 2 replicates at 7 depths and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatments.

Table 3.4. Soil chemical characterization at Birch Mountain by treatment, merged 0 to 70 cm depth, August 2000.

Characteristic	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	SE
Available Nitrate ($mg\ kg^{-1}$)	1.2 a	13.5 b	1.0 a	2.4 a	2.4
Available Phosphate ($mg\ kg^{-1}$)	2 a	2 a	3 a	2 a	1
Available Potassium ($mg\ kg^{-1}$)	18 a	28 b	26 b	33 b	2
Available Sulfate ($mg\ kg^{-1}$)	2 a	7 b	8 b	5 ab	2
Total Carbon (%)	0.7 a	0.9 a	1.4 a	1.4 a	0.4
pH	5.2 b	4.4 a	5.0 b	5.1 b	0.2
Electrical Conductivity ($mS\ cm^{-1}$)	0.18 a	0.60 b	0.17 a	0.26 a	0.09
Cation Exchange Capacity ($meq\ 100\ g^{-1}$)	5.5 a	6.3 a	4.9 a	5.4 a	0.8
Sodium Adsorption Ratio	2.6 b	1.4 a	2.5 a	1.4 a	0.3

Values represent the least squares means of 2 replicates at 7 depths and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatments.

Table 3.5. Canopy cover at Pony Creek[1] and Birch Mountain[2], August 17 to 18, 1998 and August 28 to 31, 2000.

(a) Contamination by Year

	Pony Creek					Birch Mountain				
	1998		2000			1998		2000		
Canopy Type (%)	Control	Contaminated	Control	Contaminated	SE	Control	Contaminated	Control	Contaminated	SE
Live Canopy Cover	54 b	37 a	81 c	86 c	5	39 a	39 a	74 b	70 b	7
Litter	38 c	33 c	19 b	8 a	4	19 a	42 b	12 a	15 a	5
Bare Ground	8 a	30 b	0 a	6 a	4	42 a	20 a	15 a	15 a	6

(b) Fertilizer Application by Year

	1998		2000			1998		2000		
Canopy Type (%)	Control	Fertilized	Control	Fertilized	SE	Control	Fertilized	Control	Fertilized	SE
Live Canopy Cover	47 a	44 a	75 b	92 c	5	33 a	44 a	66 b	78 b	7
Litter	43 c	28 b	24 b	3 a	4	40 b	21 a	14 a	12 a	5
Bare Ground	11 b	27 c	1 a	5 ab	4	26 bc	35 c	20 b	10 a	6

(c) Year

Canopy Type (%)	1998	2000	SE	1998	2000	SE
Live Canopy Cover	45 a	83 b	4	39 a	72 b	6
Litter	36 b	14 a	3	31 b	13 a	4
Bare Ground	19 b	3 a	3	31 b	15 a	5

[1]Values represent the least squares means of five 0.25 m^2 quadrats within 5 replicates and the standard error.
[2]Values represent the least squares means of three 0.25 m^2 quadrats within 5 replicates and the standard error.
Within a site, different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatment-years.

Table 3.6. Live canopy cover by type at Pony Creek[1] and Birch Mountain[2], August 17 to 18, 1998 and August 28 to 31, 2000.

(a) Contamination by Year

	Pony Creek					Birch Mountain				
	1998		2000			1998		2000		
Canopy Type (%)	Control	Contaminated	Control	Contaminated	SE	Control	Contaminated	Control	Contaminated	SE
Shrub/Tree	9 a	5 a	9 a	4 a	3	20 a	41 b	27 a	14 a	7
Grass/Sedge	40 a	59 b	55 b	73 c	4	35 ab	23 a	41 bc	54 c	5
Forb	47 c	29 b	30 b	17 a	4	44 c	30 bc	24 ab	19 a	6
Moss/Lichen	4 ab	1 a	6 b	5 ab	1	1 a	6 ab	8 bc	13 c	4

(b) Fertilizer Application by Year

	1998		2000			1998		2000		
Canopy Type (%)	Control	Fertilized	Control	Fertilized	SE	Control	Fertilized	Control	Fertilized	SE
Shrub/Tree	12 b	2 a	11 ab	2 a	4	35 b	26 b	31 b	9 a	7
Grass/Sedge	52 a	48 a	43 a	84 b	4	29 a	30 a	32 a	63 b	5
Forb	34 b	42 b	39 b	9 a	3	35 b	39 b	21 a	22 a	6
Moss/Lichen	2 a	3 ab	8 b	3 ab	1	1 a	6 a	16 b	6 a	3

(c) Year

Canopy Type (%)	1998	2000	SE	1998	2000	SE
Shrub/Tree	7 a	6 a	2	31 b	20 a	6
Grass/Sedge	50 a	64 b	3	29 a	48 b	3
Forb	38 b	24 a	3	37 b	22 a	5
Moss/Lichen	3 a	5 a	1	3 a	11 b	3

[1]Values represent the least squares means of five 0.25 m^2 quadrats within 5 replicates and the standard error.
[2]Values represent the least squares means of three 0.25 m^2 quadrats within 5 replicates and the standard error.
Within a site, different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatment-years.

Table 3.7. Canopy cover at Pony Creek[1] and Birch Mountain[2] by treatment and year, August 17 to 18, 1998 and August 28 to 31, 2000.

Canopy Type (%)	1998 Control Unfertilized	1998 Control Fertilized	1998 Contaminated Unfertilized	1998 Contaminated Fertilized	2000 Control Unfertilized	2000 Control Fertilized	2000 Contaminated Unfertilized	2000 Contaminated Fertilized	SE
<u>Pony Creek</u>									
Live Canopy Cover	52 bc	54 bc	41 ab	34 a	64 c	98 d	86 d	86 d	6
Litter	42 c	34 c	43 c	22 b	37 c	2 a	12 ab	4 a	4
Bare Ground	5 ab	11 ab	16 b	44 c	0 a	0 a	2 a	10 ab	6
<u>Birch Mountain</u>									
Live Canopy Cover	38 ab	39 ab	29 a	49 bc	69 d	79 d	64 c	77 d	8
Litter	32 b	7 a	48 c	36 bc	13 a	10 a	16 a	15 a	6
Bare Ground	30 b	54 c	23 ab	16 ab	18 ab	11 a	21 ab	8 a	7

[1]Values represent the least squares means of five 0.25 m^2 quadrats within 5 replicates and the standard error.
[2]Values represent the least squares means of three 0.25 m^2 quadrats within 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatments.

Table 3.8. **Live canopy cover by type at Pony Creek[1] and Birch Mountain[2] by treatment and year, August 17 to 18, 1998 and August 28 to 31, 2000.**

Canopy Type (%)	1998 Control Unfertilized	1998 Control Fertilized	1998 Contaminated Unfertilized	1998 Contaminated Fertilized	2000 Control Unfertilized	2000 Control Fertilized	2000 Contaminated Unfertilized	2000 Contaminated Fertilized	SE
Pony Creek									
Shrub/Tree	14 b	3 ab	10 ab	0 a	14 b	4 ab	8 ab	4 ab	4
Grass/Sedge	41 b	40 ab	62 c	56 c	26 a	83 d	59 c	82 d	5
Forb	42 cd	52 d	26 b	32 bc	48 cd	12 a	29 b	5 a	4
Moss/Lichen	3 a	5 a	2 a	0 a	12 b	1 a	4 a	5 a	2
Birch Mountain									
Shrub/Tree	26 bcd	14 ab	44 d	37 cd	39 bcd	14 ab	22 abc	5 a	9
Grass/Sedge	26 ab	45 cd	32 bc	14 a	24 ab	58 de	40 bc	68 e	6
Forb	47 c	41 c	24 ab	36 bc	21 ab	27 ab	21 ab	17 a	7
Moss/Lichen	1 ab	0 a	0 a	11 c	15 c	1 ab	17 c	10 bc	4

[1]Values represent the least squares means of five 0.25 m^2 quadrats within 5 replicates and the standard error.
[2]Values represent the least squares means of three 0.25 m^2 quadrats within 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatments.

Table 3.9. Percentage live canopy cover of major taxa at Pony Creek by contamination and year, August 17 to 18, 1998 and August 28 to 31, 2000.

	1998		2000		
Canopy Type (%)	Control	Contaminated	Control	Contaminated	SE
Achillea millifolium	2 ab	1 a	2 b	1 a	1
Agrostis scabra	3 a	5 a	3 a	28 b	3
Aster spp.	17 c	10 b	6 ab	4 a	2
Betula spp.	2 b	1 ab	0 a	0 a	1
Carex spp.	3 a	4 a	11 b	15 b	3
Epilobium spp.	10 bc	3 a	16 c	2 a	3
Festuca rubra	32 bc	21 ab	37 c	12 a	4
Lycopodium spp.	1 a	0 a	3 b	0 a	1
Poa spp.	1 a	27 c	3 a	15 b	4
Potentilla tridentata	3 a	2 a	1 a	0 a	1
Salix spp.	2 a	3 a	2 a	2 a	2
Trifolium hybridum	8 b	3 ab	1 a	6 ab	2
Vaccinium spp.	1 ab	0 a	3 b	0 a	1
Taxa Richness	5 b	4 ab	4 a	4 a	1

Taxa were included which had ≥ 5% live canopy cover in any treatment in either year.
Taxa richness includes all taxa.
Values represent the least squares means of five 0.25 m^2 quadrats within 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatments.

Table 3.10. Percentage live canopy cover of major taxa at Birch Mountain by contamination and year, August 17 to 18, 1998 and August 28 to 31, 2000.

	1998		2000		
Canopy Type (%)	Control	Contaminated	Control	Contaminated	SE
Agrostis scabra	5 ab	0 a	13 b	12 b	4
Alnus crispa	5 a	9 ab	18 b	1 a	5
Arctostaphylos uva-ursi	1 a	7 b	0 a	0 a	1
Betula spp.	4 ab	0 a	6 b	0 a	1
Calamagrostis spp.	10 b	1 a	3 a	0 a	2
Carex spp.	2 a	1 a	1 a	29 b	4
Cornus canadensis	0 a	6 b	0 a	2 a	2
Cornus stolonifera	0 a	8 a	0 a	3 a	4
Epilobium spp.	18 b	17 b	5 a	6 a	4
Festuca rubra	15 b	14 b	4 a	10 ab	4
Fragaria virginiana	0 a	5 a	2 a	2 a	3
Plantago major	5 b	0 a	2 a	1 a	1
Poa spp.	1 a	0 a	20 b	1 a	2
Rubus spp.	10 b	4 ab	4 a	1 a	3
Salix spp.	7 b	4 ab	2 ab	0 a	2
Taraxacum officinale	4 b	0 a	7 c	1 a	1
Vaccinium spp.	0 a	11 a	1 a	9 a	4
Taxa Richness	6 b	4 a	6 b	5 a	1

Taxa were included which had ≥ 5% live canopy cover in any treatment in either year.
Taxa richness includes all taxa.
Values represent the least squares means of three 0.25 m^2 quadrats within 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatments.

Table 3.11. Percentage live canopy cover of major taxa at Pony Creek by fertilizer application and year, August 17 to 18, 1998 and August 28 to 31, 2000.

	1998		2000		
Canopy Type (%)	Control	Fertilized	Control	Fertilized	SE
Achillea millifolium	1 b	1 b	3 c	0 a	1
Agrostis scabra	1 a	6 a	6 a	25 b	3
Aster spp.	13 b	13 b	9 ab	0 a	2
Betula spp.	3 b	0 a	0 a	0 a	1
Carex spp.	6 a	1 a	23 b	2 a	3
Epilobium spp.	10 b	3 a	10 b	8 ab	2
Festuca rubra	28 b	25 b	7 a	42 c	4
Lycopodium spp.	1 a	0 a	3 b	0 a	1
Poa spp.	13 b	15 b	4 a	14 b	4
Potentilla tridentata	0 a	4 b	1 ab	0 a	1
Salix spp.	5 a	1 a	2 a	2 a	2
Trifolium hybridum	3 a	8 b	7 ab	0 a	2
Vaccinium spp.	0 ab	1 ab	3 b	0 a	1
Taxa Richness	5 c	4 b	6 c	2 a	1

Taxa were included which had ≥ 5% live canopy cover in any treatment in either year.
Taxa richness includes all taxa.
Values represent the least squares means of five 0.25 m^2 quadrats within 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatments.

Table 3.12. Percentage live canopy cover of major taxa at Birch Mountain by fertilizer application and year, August 17 to 18, 1998 and August 28 to 31, 2000.

	1998		2000		
Canopy Type (%)	Control	Fertilized	Control	Fertilized	SE
Agrostis scabra	4 a	2 a	7 a	18 b	4
Alnus crispa	6 a	8 a	13 a	5 a	5
Arctostaphylos uva-ursi	2 a	6 b	0 a	0 a	1
Betula spp.	4 ab	0 a	5 b	0 a	1
Calamagrostis spp.	1 a	10 b	0 a	3 a	2
Carex spp.	1 a	1 a	19 b	11 ab	4
Cornus canadensis	5 a	1 a	1 a	0 a	2
Cornus stolonifera	7 b	2 ab	3 ab	0 a	3
Epilobium spp.	13 a	22 b	5 a	6 a	3
Festuca rubra	15 b	13 b	2 a	12 b	3
Fragaria virginiana	5 a	0 a	2 a	2 a	3
Plantago major	3 a	2 a	3 a	0 a	1
Poa spp.	0 a	1 a	3 a	17 b	3
Rubus spp.	6 a	8 a	2 a	3 a	2
Salix spp.	10 b	2 a	0 a	2 a	2
Taraxacum officinale	1 a	3 ab	3 ab	5 b	1
Vaccinium spp.	8 a	4 a	9 a	2 a	4
Taxa Richness	6 ab	5 ab	6 b	4 a	1

Taxa were included which had ≥ 5% live canopy cover in any treatment in either year.
Taxa richness includes all taxa.
Values represent the least squares means of three 0.25 m^2 quadrats within 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatments.

Table 3.13. Percentage live canopy cover of major taxa at Pony Creek by Year, August 17 to 18, 1998 and August 28 to 31, 2000.

Canopy Type (%)	1998	2000	SE
Achillea millifolium	1 a	2 a	1
Agrostis scabra	4 a	15 b	2
Aster spp.	13 b	5 a	2
Betula spp.	2 b	0 a	1
Carex spp.	4 a	13 b	2
Epilobium spp.	7 a	9 a	2
Festuca rubra	27 a	25 a	3
Lycopodium spp.	1 a	2 a	1
Poa spp.	14 a	9 a	3
Potentilla tridentata	2 a	1 a	1
Salix spp.	3 a	2 a	1
Trifolium hybridum	5 a	3 a	1
Vaccinium spp.	0 a	1 a	1
Taxa Richness	5 b	4 a	1

Taxa were included which had ≥ 5% live canopy cover in any treatment in either year.
Taxa richness includes all taxa.
Values represent the least squares means of five 0.25 m^2 quadrats within 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) between treatments.

Table 3.14. Percentage live canopy cover of major taxa at Birch Mountain by year, August 17 to 18, 1998 and August 28 to 31, 2000.

Canopy Type (%)	1998	2000	SE
Agrostis scabra	2 a	12 b	3
Alnus crispa	7 a	9 a	3
Arctostaphylos uva-ursi	4 b	0 a	1
Betula spp.	2 a	3 a	1
Calamagrostis spp.	6 a	2 a	2
Carex spp.	1 a	15 b	3
Cornus canadensis	3 a	1 a	1
Cornus stolonifera	4 a	2 a	3
Epilobium spp.	17 b	6 a	3
Festuca rubra	14 b	7 a	3
Fragaria virginiana	3 a	2 a	2
Plantago major	3 a	2 a	1
Poa spp.	1 a	10 b	2
Rubus spp.	7 b	2 a	2
Salix spp.	6 b	1 a	1
Taraxacum officinale	2 a	4 a	1
Vaccinium spp.	6 a	5 a	3
Taxa Richness	5 a	5 a	1

Taxa were included which had ≥ 5% live canopy cover in any treatment in either year.
Taxa richness includes all taxa.
Values represent the least squares means of three 0.25 m^2 quadrats within 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) between treatments.

Table 3.15. Percentage live canopy cover of major taxa at Pony Creek by treatment and year, August 17 to 18, 1998 and August 28 to 31, 2000.

Taxa	1998				2000				SE
	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	
Achillea millefolium	2 a	2 a	1 a	1 a	5 b	0 a	2 a	0 a	1
Agrostis scabra	2 a	3 a	1 a	9 a	3 a	3 a	8 a	47 b	4
Aster spp.	15 cd	18 d	12 bcd	8 bc	12 bcd	0 a	7 ab	0 a	3
Betula spp.	5 b	0 a	3 ab	0 a	0 a	0 a	0 a	0 a	1
Carex spp.	5 a	0 a	6 a	3 a	18 b	3 a	28 c	2 a	3
Epilobium spp.	17 c	3 a	2 a	3 ab	20 c	12 bc	0 a	3 ab	3
Festuca rubra	29 d	35 d	27 cd	15 bc	2 a	72 e	12 ab	12 ab	5
Lycopodium spp.	2 a	0 a	0 a	0 a	7 b	0 a	0 a	0 a	1
Poa spp.	0 a	2 a	26 c	28 c	0 a	6 a	9 ab	21 bc	5
Potentilla tridentata	0 a	5 b	0 a	4 ab	2 ab	0 a	0 a	0 a	2
Salix spp.	3 ab	1 ab	6 b	0 a	0 a	3 ab	4 ab	0 a	2
Trifolium hybridum	4 a	12 b	1 a	4 a	1 a	0 a	12 b	0 a	3
Vaccinium spp.	0 a	1 a	0 a	0 a	5 b	0 a	0 a	5 b	1
Taxa Richness	5 c	5 cd	6 d	3 b	5 cd	2 ab	6 d	2 a	1

Taxa were included which had ≥ 5% live canopy cover in any treatment in either year.
Taxa richness includes all taxa.
Values represent the least squares means of five 0.25 m^2 quadrats within 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatments.

Table 3.16. Percentage live canopy cover of major taxa at Birch Mountain by treatment and year, August 17 to 18, 1998 and August 28 to 31, 2000.

Taxa	1998				2000				SE
	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	
Agrostis scabra	8 a	3 a	0 a	0 a	12 ab	13 ab	0 a	23 b	5
Alnus crispa	6 a	4 a	7 a	12 ab	26 b	9 ab	0 a	1 a	7
Arctostaphylos uva-ursi	2 a	0 a	1 a	13 b	0 a	0 a	0 a	0 a	2
Betula spp.	7 b	0 a	0 a	0 a	10 b	0 a	0 a	0 a	2
Calamagrostis spp.	1 a	19 b	1 a	0 a	0 a	7 a	0 a	0 a	3
Carex spp.	3 a	0 a	0 a	1 a	2 a	0 a	36 c	21 b	5
Cornus canadensis	0 a	0 a	9 b	3 a	0 a	0 a	3 a	0 a	2
Cornus stolonifera	0 a	0 a	14 b	3 a	0 a	0 a	7 ab	0 a	4
Epilobium spp.	21 b	15 ab	4 a	28 b	4 a	6 a	6 a	5 a	5
Festuca rubra	10 ab	19 b	20 b	7 a	3 a	3 a	0 a	20 b	4
Fragaria virginiana	0 a	0 a	10 b	0 a	0 a	4 ab	3 a	1 a	4
Plantago major	6 b	5 b	0 a	0 a	4 ab	1 a	2 ab	0 a	2
Poa spp.	0 a	1 a	0 a	1 a	6 a	34 b	0 a	1 a	3
Rubus spp.	9 ab	11 b	3 ab	5 ab	4 ab	5 ab	0 a	1 a	4
Salix spp.	12 c	3 ab	7 bc	1 ab	0 a	4 ab	0 a	0 a	3
Taraxacum officinale	2 ab	5 bc	1 a	0 a	5 bc	9 c	1 a	1 a	2
Vaccinium spp.	1 a	0 a	15 b	8 ab	2 ab	0 a	15 b	3 ab	6
Taxa Richness	7 d	5 bc	4 ab	5 bc	7 cd	5 bcd	6 cd	4 a	1

Taxa were included which had ≥ 5% live canopy cover in any treatment in either year.
Taxa richness includes all taxa.
Values represent the least squares means of three 0.25 m^2 quadrats within 5 replicates and the standard error.
Different letters in a row indicate statistically significant differences (LSD, $p \leq 0.05$) among treatments.

4. Future Directions For Reclamation of Diesel Fuel Contaminated Sites

4.1 Introduction

Although research related to reclamation of diesel fuel and other hydrocarbon contaminated sites has been conducted for more than thirty years, significant gaps remain in understanding the complex interactions between diesel fuel contamination, fertilizer application, soil microorganisms, vegetation and site-specific biotic and abiotic factors. Each study that is conducted, including this research, increases our base of knowledge and exposes the limits of our understanding. Within major research foci, this chapter briefly summarizes the current state of knowledge, contributions of this study and potential future research directions.

4.2 Biostimulation

The ability of some soil microorganisms to degrade hydrocarbons has been recognized for several decades. An early (Jobson et al. 1972, Jobson et al. 1974, Westlake et al. 1978) and ongoing (Margesin and Schinner 2001) research focus has been enhancement of this process to reduce the timeframes required for reclamation. Nitrogen and phosphorus supplements are often utilized to re-establish the balance of soil nutrient levels and stimulate biodegradation of the contaminant hydrocarbons.

Recommended levels of nitrogen and phosphorus in soil for microorganisms vary from C:N:P of 100:60:6 to 100:0.5:0.1 (Wang et al. 1990, Evans et al. 1991, Harder et al. 1991, Huessemann and Moore 1993, Faessler et al. 1994, Mills and Frankenburger 1994, Margesin and Schinner 1997, Kirchmann and Ewnetu 1998, Graham et al. 1999). No recommended rates of fertilizer application for plants for biostimulation or phytoremediation were identified. In this study, higher rates of fertilizer application were utilized than are typically recommended for either microorganisms or vegetation. It appears likely that tolerance levels are higher than previously anticipated. Investigation of fertilizer application rates to determine optimal levels is recommended.

The composition of fertilizer for biostimulation is typically not specified in studies. Positive results have been reported with a 27-27-0 (Jobson et al. 1972, Jobson et al. 1974, Westlake et al. 1978) and a 15-15-15 (Margesin and Schinner 2001) N-P-K mixture. During this research, 20-3-4, 34-0-0 and a mixture of 34-0-0 and 11-51-0 (N:P:K) were successfully utilized. Results during the year following the mixed application were highest, however, soil moisture may have contributed to this effect. Research studies to determine the most appropriate fertilizer or fertilizer blend are recommended.

Hydrocarbon contamination shifts the population and species diversity of microorganisms (Perry and Scheld 1968, Odu 1978, Jobson et al. 1979, Pinholt et al. 1979, Reynolds et al. 1999a). No studies were identified which considered impacts of fertilizer application on microorganism species diversity. In this study, fertilizer application increased microorganism populations and reduced microorganism diversity. Additional studies to determine the effects on population and diversity of microorganisms in contaminated soil with varying rates and types of fertilizer application should be conducted. Long-term studies to assess the impact on microorganisms after fertilizer application is discontinued should also be initiated.

4.3 Phytotolerance

The impact of hydrocarbons on vegetation was recognized in early studies (McGill and Nyborg 1975, McGill et al. 1981). Recent research has focused on species and genotypes within species of plants that are more tolerant of hydrocarbon contamination (Rogers et al. 1996, Chaineau et al. 1997, Xu and Johnson 1997, Wiltse et al. 1998, Adam and Duncan 1999, Porta et al. 1999, Reynolds et al. 1999a, Reynolds et al. 1999b). No research was identified which studied impacts of hydrocarbons or fertilizer application on plant species richness. In this study, hydrocarbon contamination reduced canopy cover; fertilizer application increased live canopy cover and reduced plant species richness. Additional studies to determine the effects on canopy cover and species richness on contaminated soil with varying rates and types of fertilizer application should be conducted.

In this study, changes in plant community composition were evident a year prior to significant changes in hydrocarbon contamination levels in the soil. This observation may assist in explanation of a perceived delayed response in reduction of hydrocarbon levels. Further investigation of the interactions between plant and microorganism community composition, plant canopy cover, microorganism populations and hydrocarbon levels should be considered.

In a typical reclamation sequence of hydrocarbon contamination, reduction of contamination levels precedes revegetation. Establishment of vegetation is generally not initiated until hydrocarbon levels are below regulatory levels. In this study and in observations on many similar sites, berry shrubs and some forbs and grasses have established in soil with levels of contamination exceeding 20,000 ppm. The possibility of initiating revegetation at an earlier stage of reclamation requires additional investigation. Long-term studies to assess the impact on vegetation after fertilizer application is discontinued should also be initiated.

4.4 Phytoremediation

The potential for direct uptake and degradation of hydrocarbons by vegetation has been investigated in some recent studies and remains uncertain. Wild and Jones (1992) confirmed uptake of sewage sludge hydrocarbons in carrots. Chaineau et al. (1997) demonstrated no uptake of hydrocarbons at levels of hydrocarbon contamination in soil sufficiently low for plant survivability; however, high concentrations of hydrocarbons were identified in the leaves and stems of plants which did not survive high levels of contamination. Banks et al. (1999) and Reynolds et al. (1999a) traced minimal carbon uptake into plants. Pichtel and Liskanen did not detect any hydrocarbons in grasses and legumes grown on diesel fuel contaminated soil. Additional investigations are required, particularly for species that demonstrate a tolerance for hydrocarbon contamination and increase percentage of live canopy cover with fertilizer application, e.g. *Agrostis scabra* and *Festuca rubra*.

4.5 Rhizosphere Bioremediation

The current research focus is on enhanced rates of biodegradation in the presence of vegetation (Reynolds et al. 1997, Reynolds et al. 1999a, Reynolds et al. 1999b, Hutchinson et al. 2001, Pichtel and Liskanen 2001). In studies of the effects of vegetation on biodegradation of hydrocarbons in soil, where bacteria numbers were determined, they were higher within the rhizosphere than in bulk soil (Lee and Banks 1993, Gunther et al. 1996, Komisar et al. 1997, Nicols et al. 1997, Reynolds et al. 1999). Recent studies have begun to examine bacterial diversity in the rhizosphere (Reynolds et al. 1999, Miya and Firestone 2000, Yang and Crowley 2000. Reynolds et al. (1999) identified an increase in bacterial species richness as hydrocarbon levels increased. Yang and Crowley (2000) determined that bacterial communities are different in the root zones within the rhizosphere. Miya and Firestone (2000) indicated that hydrocarbon degraders in the rhizosphere were less diverse than in bulk soil. Interactions and probable synergistic effects between microorganisms and vegetation require additional investigation. Ideally, some of these studies would consider mixed populations over a period of years under variable conditions, either as greenhouse simulations or field investigations.

4.6 Conclusions

There is a long history of research related to reclamation of diesel fuel contaminated sites, however, potential synergistic effects of soil microorganisms and vegetation provide new directions for investigation. Determination of most effective fertilizer combinations, optimal application rates and evaluation of long term impacts of fertilizer application on soil microorganisms and vegetation is required.

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Appendix A

Site Diagrams

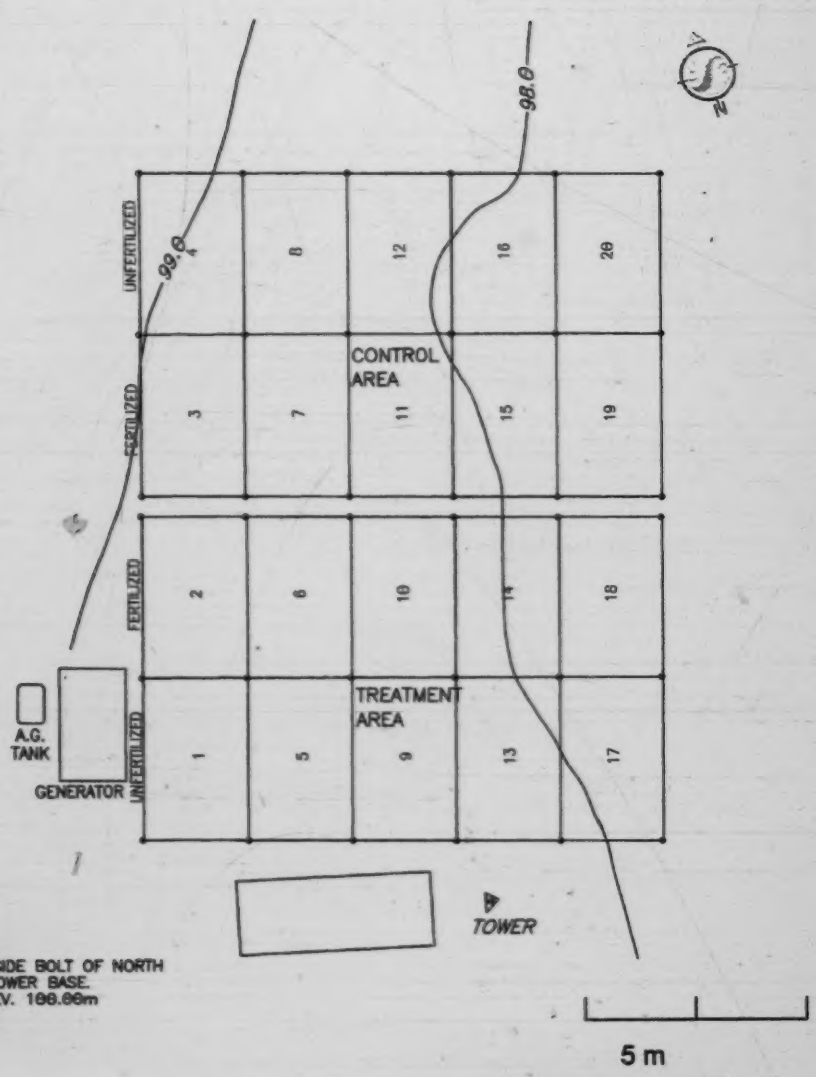
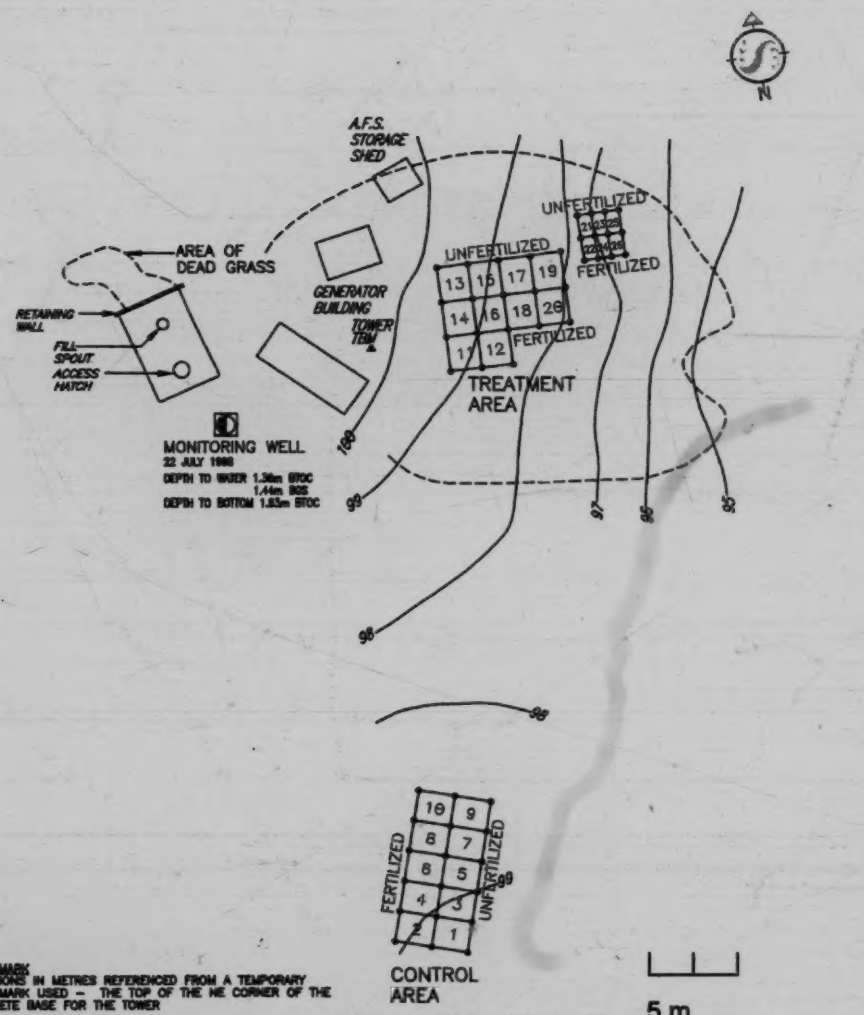


Figure A.1 Pony Creek site diagram.



Legend

MONITORING WELL

EXTENT OF SURFACE PLUME (DOWN TO 0.7m DEPTH) AS DETECTED BY SMELL

Note

SIZE AND DISTANCES ARE APPROXIMATE

Stantec

\18214766\18214731\FIG2.DWG
December 2, 1998

Client/Project
TELUS CORPORATION
SITE ASSESSMENT
BIRCH MOUNTAIN, ALBERTA

Figure No.
2

Title
Borehole and Sample Location Plan

Figure A.2 Birch Mountain site diagram.

Appendix B

Methods Descriptions

B.1 Standard Operating Procedure – Determination of Total Extractables in Soil or Sediment using the Shake Method.

B.1.1 Purpose

This method is applicable to the determination of total extractable hydrocarbons in soil/sediment samples.

B.1.2 Principle

A sample volume of ~25 g weighed in a 25 x 150 mm culture tube is vortexed, shaken and sonicated for 15 minutes with 10 ml of 1:1 hexane/acetone. The hexane/acetone layer is drawn off and analyzed using a gas chromatograph equipped with a flame-ionization detector. The limit of detection for total extractables is 5.0 ppm. The linear range for the instrument is approximately 5-5000 ppm. For accuracy and precision data, refer to matrix spike and sample duplicate control chart data.

B.1.3 Method Reference

Modified EPA SW846 methods 3550 or 3580 and 8000

Table B.1. Quality control requirements for determination of total extractables in soil or sediment using the shake method.

Quality Control Check	Frequency[1]	Acceptance Criteria (95% Confidence Interval)	Corrective Action
Calibration Curve	Daily – 3 levels	$R^2 \geq 0.995$	1. Recalibrate 2. Prepare fresh standards
Alternate Source	Daily, with calibration curve	as per Quality Control chart	3. Recalibrate 4. Prepare fresh standards
Verification standard	Every 15 samples	± 15% of predicted response	Recalibrate
Method Blank	Every batch	< Method Detection Limit	Reanalyze
Duplicates	5%	as per Quality Control chart	1. Recalculate 2. Reanalyze 3. Reextract
Matrix spikes	5%	as per Quality Control chart	1. Recalculate 2. Reanalyze 3. Reextract
Surrogate Recovery	On all samples, spikes, duplicates & method blanks	as per Quality Control chart	1. Recalculate 2. Reanalyze 3. Reextract

[1] Note additional QC may be required as defined by client request.

B.2 Soil Chemical Characterization Methodology

B.2.1 Available Nitrate, Nitrite, Nitrogen

Available Nitrate and Nitrite are extracted from the soil using a dilute calcium chloride solution. Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is measured at colorimetrically at 520nm.

Reference:
Carter, M.D. (ed.). 1993. Soil sampling and methods of analysis. Lewis Publishers. Boca Raton, Florida. 823 pp.

B.2.2 Available Phosphorous

Available orthophosphate P is extracted from the soil using Modified Kelowna extracting solution (0.025M HOAc, 0.25M NH4Oac, 0.015M NH4F at pH 4.5). The orthophosphate ion reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex that is measured colorimetrically by auto analysis at 880 nm.

Reference:
Communications in Soil Science and Plant Analysis. 25(5 and 6): 627-635 (1994).

B.2.3 Available Potassium

Available potassium is extracted from the soil using Modified Kelowna extracting solution (0.025M HOAc, 0.25M NH4Oac, 0.015M NH4F at pH 4.5). The extract is mixed with lithium nitrate, nitric acid and lanthanum oxide as an internal standard and passed into the burner of a flame photometer. Intensity of light emitted is measured at 768 nm.

Reference:
Communications in Soil Science and Plant Analysis. 25(5 and 6): 627-635 (1994).

B.2.4 Available Sulfur

The soil is extracted with a weak calcium chloride solution. The calium chloride serves to reduce the extraction of organic materials and increases flocculation of the soil in the extract. Total sulfur in the extract is then determined by ICP-AES, which is considered to be equivalent to the plant available sulfur for mineral soils from the prairies.

Reference:
Alberta Agriculture. 1988. Recommended methods of soil analysis for Canadian prairie agricultural soils. Pp. 28.

B.2.5 Saturation Paste Soil pH

Deionized water is added to the soil until the soil is saturated, but not over saturated (i.e. no free standing water). The paste is allowed to stand overnight or a minimum of four hours. The pH of the soil paste is then measured using a pH meter.

Reference:
Carter, M.D. (ed.). 1993. Soil sampling and methods of analysis. Lewis Publishers. Boca Raton, Florida. Pp. 141-142.

B.2.6 Saturation Paste Soil Conductivity

Deionized water is added to the soil until the soil is saturated, but not over saturated (ie. no free standing water). The paste is allowed to stand overnight or a minimum of four hours. After equilibration, an extract is obtained by vacuum filtration. Conductivity of the extract is measured by a conductivity meter.

Reference:
Carter, M.D. (ed.). 1993. Soil sampling and methods of analysis. Lewis Publishers. Boca Raton, Florida. Pp. 141-142.

B.2.7 Soil Saturation Paste (Ca, Mg, Na, K) used to Calculate Sodium Adsorption Ratio

Deionized water is added to the soil until the soil is saturated, but not over saturated (ie. no free standing water). The paste is allowed to stand overnight or a minimum of four hours. After equilibration, an extract is obtained by vacuum filtration. Individual cations in the extract are determined by ICP-AES.

Reference:
Carter, M.D. (ed.). 1993. Soil sampling and methods of analysis. Lewis Publishers. Boca Raton, Florida. Pp. 162-164.

B.2.8 Cation Exchange Capacity

This method involves saturation of the soil cation exchange sites with ammonium. Excess ammonium is removed from the soil with alcohol. Ammonium on the cation exchange site is then removed by leaching with NaCl and determined by autoanalyzer. This value is used to estimate cation exchange capacity.

Reference:
McKeague, J.A. 1978. Soil sampling and methods of analysis. Canadian Society of Soil Science. Pp. 78-80.

B.3 Determination of Microorganism Populations

Determine microorganism populations by type of microorganism (Jobson 1999):

1. Prepare agar plates for estimating total heterotrophic bacteria up to two weeks prior to pouring plates:
   - calculate quantity of medium required - 15ml per plate, 16 plates per sample - this provides 4 replicates at 4 levels of dilution, add 10% to calculated value for spare plates
   - purchase commercial plate count agar (e.g. from Fisher Scientific) with 1.5% agar added
   - prepare medium per manufacturer instructions, 1 litre of solution per 2 litre flask
   - autoclave medium in the flasks for 30 minutes at 15 psi and 121°C
   - allow flasks to cool until solution ceases to bubble, approximately 15 minutes
   - pour 15 ml of solution in each plate, store at room temperature upside down in the original sleeves and boxes

2. Prepare tubes for estimating iron reducing bacteria up to two weeks prior to pouring plates:
   - calculate quantity of medium required - 15ml per tube, 30 tubes per sample - this allows 5 replicates for MPN counts at 6 levels of dilution, add 10% to calculated value for spare tubes
   - prepare growth medium - recipe is calculated for 1000 ml, adjust quantity as required:
   - 0.8 grams K_2HPO_4
   - 0.2 grams KH_2PO_4
   - 0.2 grams $MgSO_4$-7H2O
   - trace NaCl
   - trace $MnSO_4$
   - trace Na_2MoO_4
   - 10 ml of saturated solution $CaSO_4$
   - 5.0 grams yeast extract
   - 5.0 grams peptone
   - 4.7 grams ferric ammonium phosphate
   - 1000 ml distilled water
   - pour 15ml into each 18 x 150mm glass tube and cap the tube
   - autoclave tubes for 30 minutes at 15 psi and 121°C
   - store tubes at room temperature

3. Prepare tubes for estimating denitrifying bacteria up to two weeks prior to pouring plates:
   - calculate quantity of medium required - 15ml per tube, 30 tubes per sample - this allows 5 replicates for MPN counts at 6 levels of dilution, add 10% to calculated value for spare tubes
   - prepare growth medium - recipe is calculated for 1000 ml, adjust quantity as required:
   - 5.0 grams KNO_3
   - 3.0 grams meat extract
   - 5.0 grams peptone

- 1000 ml distilled water
- insert an inverted Durham tube into each 18 x 150mm glass tube
- pour 15ml into each tube and cap the tube
- autoclave tubes for 30 minutes at 15 psi and 121°C
- store tubes at room temperature

4. Prepare tubes for estimating sulfate reducing bacteria up to two weeks prior to pouring plates:
   - calculate quantity of medium required - 15ml per tube, 30 tubes per sample - this allows 5 replicates for MPN counts at 6 levels of dilution, add 10% to calculated value for spare tubes
   - prepare growth medium - recipe is calculated for 1000 ml, adjust quantity as required:
   - 0.5 grams K_2HPO_4
   - 1.0 gram NH_4Cl
   - 2.0 grams Na_2SO_4
   - 0.1 grams $MgSO_4$-$7H_2O$
   - 1.5 ml of 60% syrup sodium lactate
   - 1.0 gram yeast extract
   - 1000 ml distilled water
   - insert 1 shingle nail into each 18 x 150mm glass tube
   - pour 15ml into each tube and cap the tube
   - autoclave tubes for 30 minutes at 15 psi and 121°C
   - store tubes at room temperature

5. Create serial dilutions:
   - prepare and sterilize 2 100ml dilution blanks and 4 18 ml by 150 ml tubes for each soil sample
   - fill each dilution blank with 90ml of 3mm phosphate buffer or 90ml of 1% Bacto Peptone
   - fill each dilution tube with 9 ml of a buffer solution comprised of 1 litre of distilled water, 0.12g K_2HPO_4 and 0.12g of KH_2PO_4
   - cap each dilution blank and tube and autoclave for 30 minutes at 15 psi and 121°C
   - serial dilution of soil samples:
   - weigh 10g of soil into a 90ml dilution blank
   - close the blank and shake vigorously 10 times to create the 10^{-1} dilution
   - pipette 10 ml from the 10^{-1} dilution into a second dilution blank to create the 10^{-2} dilution
   - repeat the previous step with 1 ml four times to create the 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions, vortex solution between dilutions

6. Prepare plates for total aerobic heterotrophic bacteria:
   - arrange groups of 3 plates in 4 rows labeled 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}
   - innoculate as follows for depths to 30 cm:
   - 0.1ml 10^{-3} dilution on 10^{-4} plates
   - 0.1ml 10^{-4} dilution on 10^{-5} plates
   - 0.1ml 10^{-5} dilution on 10^{-6} plates
   - 0.1ml 10^{-6} dilution on 10^{-7} plates
   - innoculate as follows for depths from 30 to 70 cm:

- 0.1ml 10^{-2} dilution on 10^{-3} plates
- 0.1ml 10^{-3} dilution on 10^{-4} plates
- 0.1ml 10^{-4} dilution on 10^{-5} plates
- 0.1ml 10^{-5} dilution on 10^{-6} plates
- spread innoculum over the agar surface of the plate with a sterile glass "hockey stick"
- incubate with the lid down at room temperature for 10 days

7. Prepare tubes for iron reducing bacteria, denitrifying bacteria and sulfate reducing bacteria
   - arrange groups of 5 tubes in 5 rows labeled 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} for each type of bacteria
   - innoculate tubes with 1ml from the corresponding dilution tube, replace lids
   - incubate tubes at room temperature for up to 28 days

8. Count aerobic heterotrophic bacteria. Choose dilutions with approximately 30 to 300 colonies for counting

9. Score anaerobic bacteria in tubes:
   - for iron reducing bacteria, a positive score is a grey precipate at the bottom of the tube
   - for denitrifying bacteria, a positive score is gas production and accumulation in the inverted Durham tubes
   - for sulfate reducing bacteria, a positive score is a black (FeS) precipitate at the bottom of the culture tubes
   - score all positives against an MPN chart with added accounting for dilution

Appendix C

Additional Tables and Figures

Table C.1. Comparisons of atypical biochemical reactions of aerobic bacteria at Pony Creek 0 to 40 cm depth, August 2000, % of population.

Depth Interval (cm)	Control Unfertilized	Control Fertilized	Contaminated Unfertilized	Contaminated Fertilized	SE
Aerobic Glucose	20 a	20 a	27 a	16 a	11
Sucrose	18 b	4 ab	0 a	0 a	5
Lactose	10 ab	0 a	24 ab	29 b	9
Xylose	62 a	36 a	30 a	34 a	12
Mannitol	61 a	78 a	44 a	78 a	14
Maltose	52 a	29 a	32 a	24 a	13

Values represent the least squares means of 4 depths and the standard error.
Different letters in a row indicate statistically significant differences (LSD; $p \leq 0.05$) among treatments.

Table C.2. Vegetation taxa by site and year identified.

Latin Name[1]	Common Name[1]	Pony Creek 1998	Pony Creek 2000	Birch Mountain 1998	Birch Mountain 2000
Shrubs and Trees					
Alnus crispa (Ait.) Pursh	green alder	✓	✓	✓	✓
Arctostaphylos uva-ursi (L.) Spreng.	common bearberry, kinnikinnick			✓	
Betula L.	birch	✓		✓	✓
Cornus canadensis L.	bunchberry		✓	✓	✓
Cornus stolonifera Michx.	red osier, dogwood			✓	✓
Ledum groenlandicum Oeder	common labrador tea	✓	✓		✓
Picea glauca (Moench) Voss	white spruce				✓
Pinus contorta Loudon	lodgepole pine		✓		
Populus balsamifera L.	balsam poplar		✓	✓	
Populus tremuloides Michx.	aspen	✓		✓	
Salix L.	willow	✓	✓	✓	✓
Vaccinium L.	blueberry, bilberry	✓	✓	✓	✓
Grasses, Sedges and Rushes					
Agropyron Gaertn.	wheat grass			✓	✓
Agrostis scabra Willd.	hair grass, tickle grass	✓	✓	✓	✓
Calamagrostis Adans.	reed grass	✓	✓	✓	✓
Carex L.	sedge	✓	✓	✓	✓
Elymus innovatus Beal	hairy wild rye			✓	
Festuca rubra L.	red fescue	✓	✓	✓	✓
Juncus balticus Willd.	wire rush	✓			
Juncus vaseyi Engelm.	big-head rush[2]	✓			
Muhlenbergia Schreb.	muhly grass			✓	
Phelum pratense L.	timothy	✓	✓		
Poa L.	bluegrass	✓	✓	✓	✓
Forbs					
Achillea millefolium L.	common yarrow	✓	✓	✓	✓
Achillea sibirica Ledeb.	many-flowered yarrow[2]		✓		
Anaphalis margaritacea (L.) Benth. & Hook.	pearly everlasting		✓		
Artemisia L.	wormwood, sagebrush				✓
Aster L.	aster	✓	✓	✓	
Crepis tectorum L.	annual hawksbeard			✓	✓
Epilobium L.	willow-herb, fireweed[2]	✓	✓	✓	✓
Erigeron L.	fleabane, wild daisy	✓			

Table C.2. Vegetation taxa by site and year identified (continued).

Euphrasia arctica Lange ex Rostrup	eyebright		✓		
Fragaria virginiana Duchesne	wild strawberry	✓	✓	✓	✓
Gentianella amarella (L.) Börner	felwort, northern gentian[2]				✓
Hieracium albiflorum Hook.	white hawkweed		✓	✓	
Hieracium umbellatum L.	narrow-leaved hawkweek	✓	✓	✓	✓
Matricaria matrcarioides (Less.) Porter	pineapple weed				✓
Melilotus alba Desr.	white sweet clover	✓			
Parnassia palustris L.	northern grass-of-parnassus[2]	✓			
Pedicularis L.	lousewort		✓		
Penstemon procerus Dougl. ex Grah.	slender blue beard-tongue			✓	
Pinguicula vulgaris L.	common butterwort				✓
Plantago major L.	common plantain, whiteman's-foot	✓	✓	✓	✓
Potentilla tridentata Ait.	three-toothed cinquefoil	✓	✓	✓	✓
Rhinanthus minor L.	yellow rattle	✓	✓		
Rubus L.	raspberry, bramble	✓		✓	✓
Solidago L.	goldenrod		✓	✓	
Sonchus L.	sow thistle			✓	
Taraxacum officinale Weber	common dandilion		✓	✓	✓
Trifolium hybridum L.	alsike clover	✓	✓		✓
Trifolium repens L.	white clover, dutch clover	✓	✓		
Vicea americana Muhl.	wild vetch		✓		
Mosses, lichens, liverworts and horsetails					
Equisetum arvense L.	common or field horsetail	✓	✓		
lichen	lichen	✓	✓	✓	✓
liverwort	liverwort				✓
Lycopodium L.	club-moss	✓	✓		
moss	moss	✓	✓	✓	✓

[1] Reference for all Latin names and common names unless otherwise indicated: Moss, E.H. 1994. Flora of Alberta:A manual of flowering plants, conifers, ferns and fern allies found growing without cultivation in the province of Alberta, Canada. Second edition. Revised by J.G. Packer. University of Toronto Press. Toronto, Canada. 687 pp.

[2] Johnson, D., L. Kershaw, A. MacKinnon and J. Pojar. 1995. Plants of the western boreal forest and aspen parkland. Lone Pine Publishers. Edmonton, Alberta. 392 pp.

unfertilized 1998

Depth Interval (cm)	Plot 1	Plot 5	Plot 9	Plot 13	Plot 17
0 to 10					
10 to 20					
20 to 30					
30 to 40					
40 to 50					
50 to 60					
60 to 70					

unfertilized 2000

Depth Interval (cm)	Plot 1	Plot 5	Plot 9	Plot 13	Plot 17
0 to 10					
10 to 20					
20 to 30					
30 to 40					
40 to 50					
50 to 60					
60 to 70					

fertilized 2001

Depth Interval (cm)	Plot 1	Plot 5	Plot 9	Plot 13	Plot 17
0 to 10					
10 to 20					
20 to 30					
30 to 40					
40 to 50					
50 to 60					
60 to 70					

ppm	
	not tested
	<1000
	1000-4999
	5000-9999
	10000-19999
	>20000

Figure C.1 Hydrocarbon levels (ppm) by depth at Pony Creek, 0 to 70 cm, unfertilized 1998, unfertilized 2000, fertilized 2001.

fertilized 1998

Depth Interval (cm)	Plot 2	Plot 6	Plot 10	Plot 14	Plot 18
0 to 10					
10 to 20					
20 to 30					
30 to 40					
40 to 50					
50 to 60					
60 to 70					

fertilized 2000

Depth Interval (cm)	Plot 2	Plot 6	Plot 10	Plot 14	Plot 18
0 to 10					
10 to 20					
20 to 30					
30 to 40					
40 to 50					
50 to 60					
60 to 70					

fertilized with holes 2001

Depth Interval (cm)	Plot 2	Plot 6	Plot 10	Plot 14	Plot 18
0 to 10					
10 to 20					
20 to 30					
30 to 40					
40 to 50					
50 to 60					
60 to 70					

ppm	
	not tested
	<1000
	1000-4999
	5000-9999
	10000-19999
	>20000

Figure C.2 Hydrocarbon levels by depth at Pony Creek, 0 to 70 cm, fertilized 1998, fertilized 2000, fertilized with holes 2001.

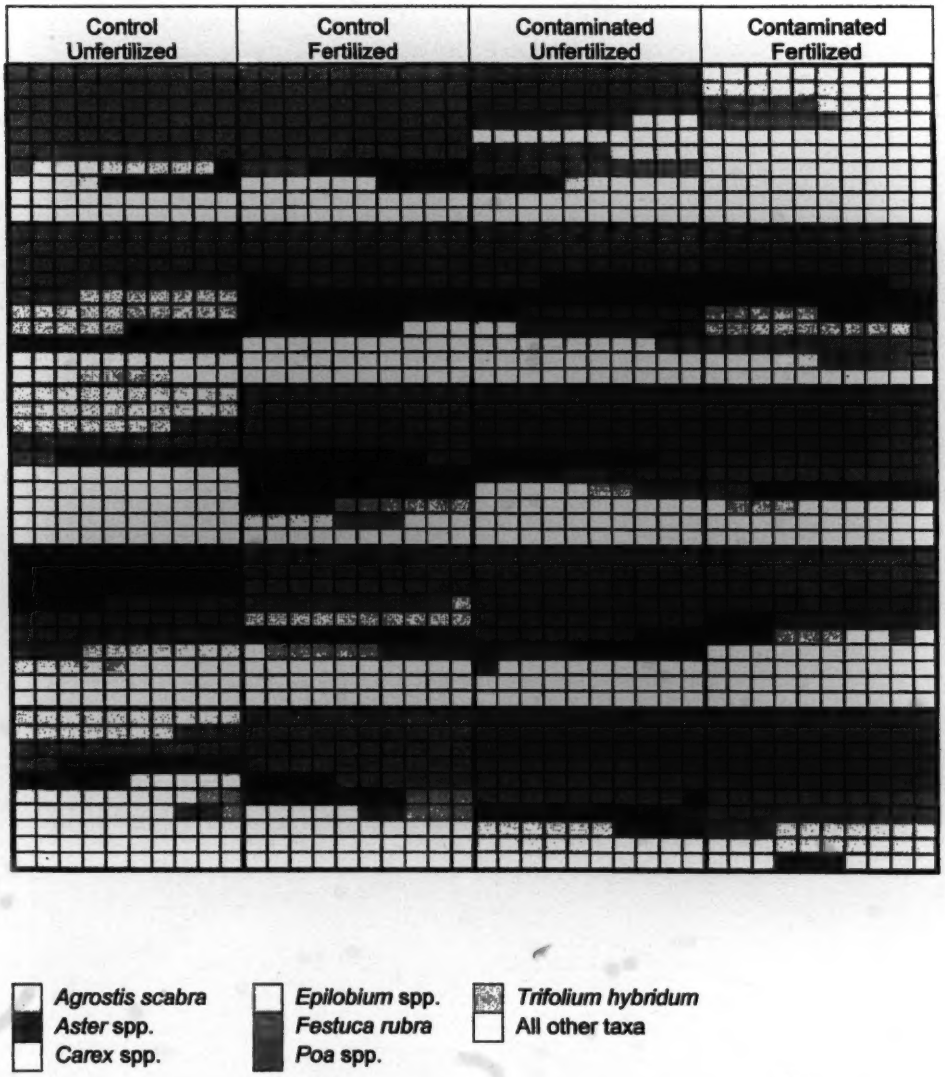


Figure C.3 **Pony Creek primary taxa live cover by treatment 1998. Each square represents 1% cover within a plot, plots are arranged by proximity to generator building.**

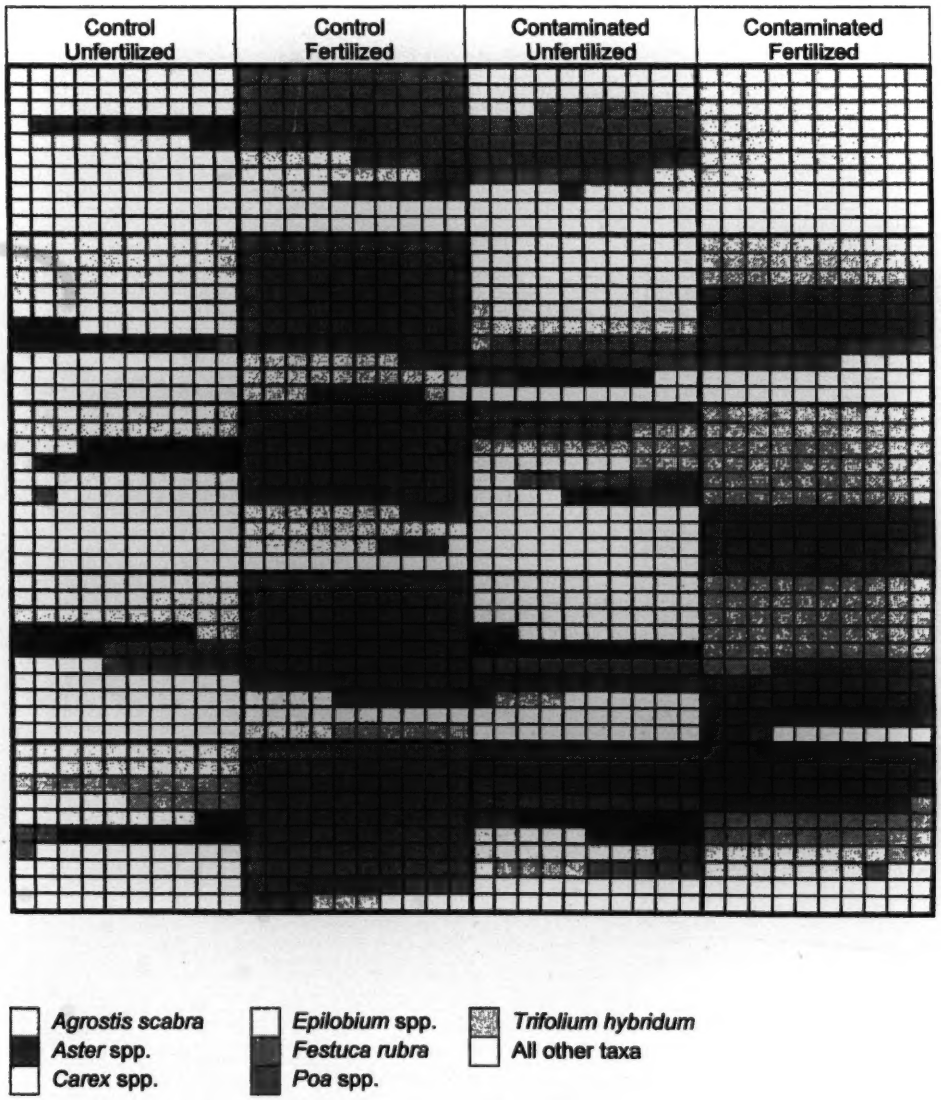


Figure C.4 **Pony Creek primary taxa live cover by treatment 2000. Each square represents 1% cover within a plot, plots are arranged by proximity to generator building.**

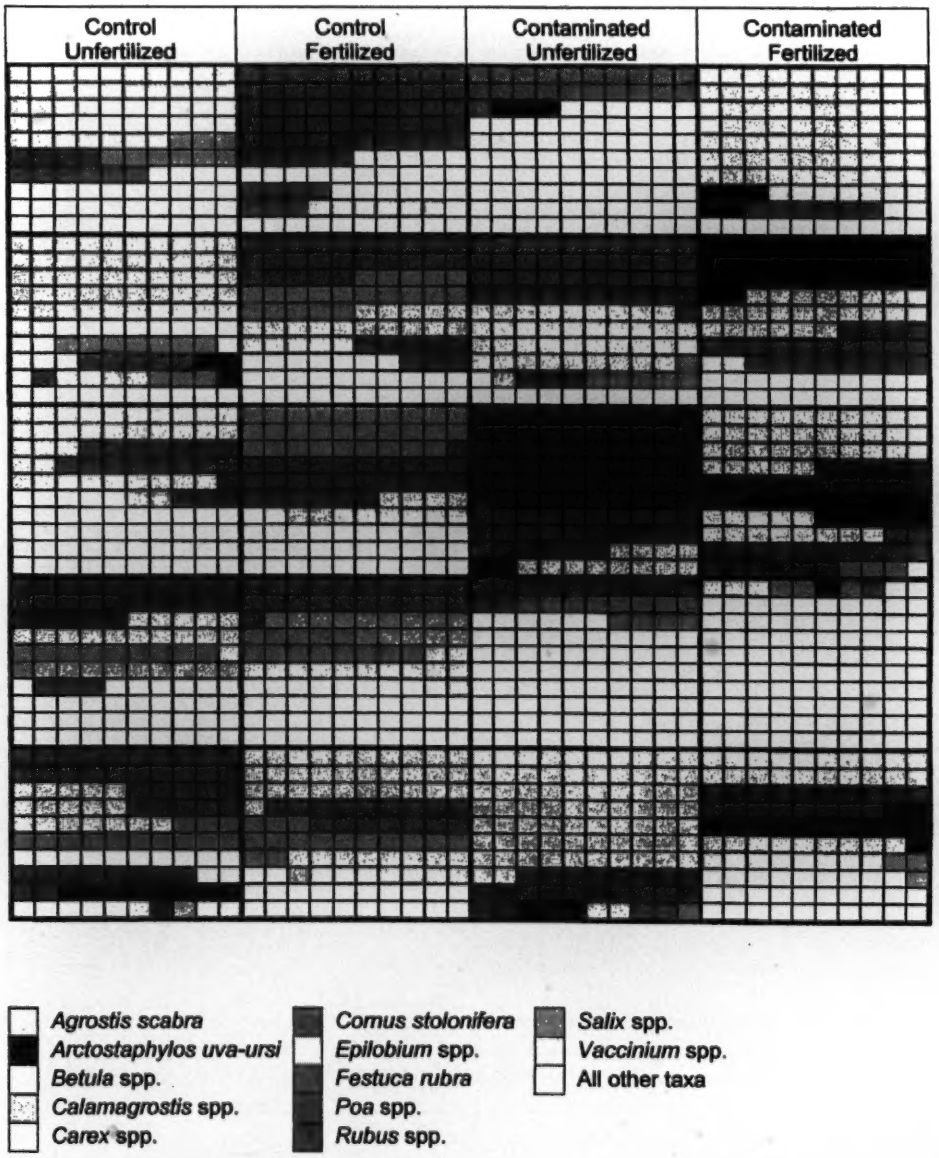


Figure C.5 **Birch Mountain primary taxa live cover by treatment 1998. Each square represents 1% cover within a plot, plots are arranged by proximity to generator building.**

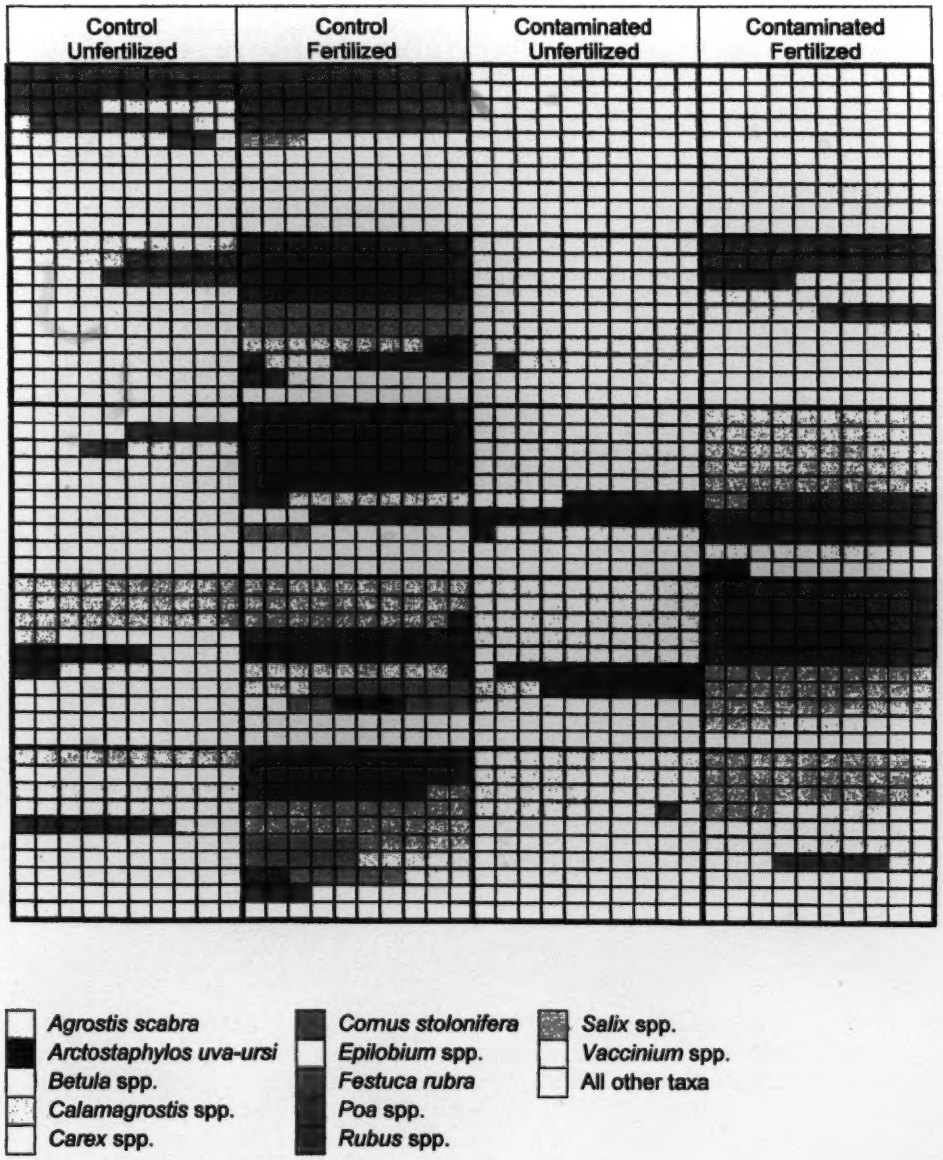


Figure C.6 **Birch Mountain primary taxa live cover by treatment 2000. Each square represents 1% cover within a plot, plots are arranged by proximity to generator building.**